The biology and macroparasites of the sixgill sawshark *Pliotrema warreni*

University of Cape Town

Department of Biological Sciences

Dissertation presented for the degree of Master of Science (MSc), Applied Marine Science

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July 2016

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Biological Sciences

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Acknowledgements

I would firstly like to thank the University of Cape Town for the remarkable opportunity to come to South Africa and spend over one year of my life studying and learning so many new things both inside and outside of the institution. Many thanks to the John Day secretarial staff whose doors were always open and who were always available to track down more supplies whenever I needed them, sometimes multiple times a day.

To the Biological Sciences department, I am indebted to you for the use of the wet lab and the lab supplies and scopes. I also owe a debt of gratitude to the Applied Marine Science program and the people within it who make it all possible. Without the use of the wet lab during research and the AMS lab during countless hours or learning, studying, researching, and writing, none of the success I have achieved in this program and on this thesis would have been possible. From inside the lecture room, I also wish to thank all of the lecturers who bequeathed their knowledge and experience upon me during the coursework component of this program.

To the former Applied Marine Science course convener, Ms. Pavs Pillay who was always available for assistance when I needed a question answered to sparing five or ten minutes to chat about everything that was going on and help calm the occasional fraying nerve.

The current AMS course convener and my co-supervisor on this project, Cecile. Without your dedicated service to me as my course convener and my co-supervisor the year would not have gone quite so smoothly. However, it was your guidance and willingness to a coffee or a quick bite and discuss the program, my progress on this thesis, or any other topics to distract us from work for a brief period that really provided the assistance and support necessary to finish this program and this thesis. I truly got to know a side of you that I wouldn't otherwise were it not for developing common interests both academically and extracurricular. Your strong interest and background in parasitology has not only impacted my thesis but also lent direction in my career choices in the future. Also, on a personal note, your assistance in getting my body in better shape through endless running has helped to bring about a healthy me and a clearer mind which was necessary for completing both this program and this thesis. For everything you have done for me I am truly grateful, though not only for your mentorship but also your friendship.

I would also like to thank my head supervisor, Assoc. Prof. Colin Attwood, without whom this entire project would not have been possible. Your dry sense of humor and your belief that I can take a small amount of direction and information and make work what you want made me a better and more independent student and person. I hope this dissertation does you proud.

I also extend my thanks to those from the Department of Agriculture, Forestry, and Fisheries who supplied the specimens in which this project was developed from. More importantly to Charlene da Silva who was not always around at UCT but was never more than a phone call or text message away from an answer and helpful hand.

To my fellow students in the AMS program, I would like to thank you for new friendships and everlasting memories and experiences. Also to those students outside the program like those in the Parasitos group and Raquel Flynn who was always eager and available to assist in the lab any way she could.

Finally, and perhaps most importantly, I want to thank my family and friends from back home in the United States whose unwavering support and belief in me and my ability to do anything I set my mind to, it is you lot who truly make everything I have done possible!

Abstract

Thirty-two specimens of the sixgill sawshark, *Pliotrema warreni*, were trawled near Bird Island in Algoa Bay on the Eastern coast of South Africa in April and May 2015. The specimens were examined for anatomical proportions, reproductive characteristics, diet, and parasite assemblages. Several external measurements were collected including mass, total length, standard length, girth, rostrum length, interoccular to pre-caudal length, first dorsal origin to second dorsal origin, first dorsal origin to pre-caudal origin, and mouth width. The equation for mass (g) vs. total length (mm) was ln(Mass)=0.2997*ln(TL)+2.0383 for females and ln(Mass)=0.3321*ln(TL)+1.941 for males. 1st Dorsal to 2nd dorsal origin length (DD) to total length equations for females and males were DD=0.2451*TL-26.677 and DD=0.2598*TL-34.535, respectively. Mean lengths and masses were 11.5% greater and 50.3% heavier in females than males, respectively. Females were on average, 994 mm (759 mm – 1283 mm) in length while males were 891.8 mm (763 mm – 1015 mm). Average mass for females was 1702.5 g (602.5 g – 3478.5 g) whereas males it was 1132.6 g (687 g – 1593.5 g). Based on these data both sexes display isometric growth. Males were determined to reach sexually maturity around 850 mm which is similar to that reported by Ebert *et al*., (2013) around 830 mm. Females were found to reach sexual maturity at 1000 mm which is 100 mm smaller than what is reported by Ebert *et al*., (2013). Stomach mass increased with total mass and total length regardless of sex (female R^2 = 0.507; male R^2 = 0.213 for length and female R^2 = 0.6123; male R^2 = 0996 for mass). Females consumed larger prey items in terms of mass and length as well as a higher quantity of prey than males presumably because they are the larger sex and have an increased need for nourishment to provide for pregnancy. Prey items were redeye round herring, *Etrumeus whiteheadi* (64.96% of the diet), a benthic shrimp species not identified (7.69%), and Cape horse mackerel, *Trachurus trachurus capensis* (0.85%).

Despite strict adherence to the guidelines for age determination for elasmobranchs provided in the literature, the conventional method used which involved extensive cleaning of the vertebral centra with an array of chemicals, setting and cutting in an epoxy resin, and staining for microscopy, did not yield readable results which could be used to determine the ages of these sharks. The highest abundance of parasites were found in the stomachs. Three specimens of a cymothoid isopod was found externally. Two specimens of *Ascaris* sp. nematode were found in the visceral cavity. The remaining 18 parasites consisted of three Neoechinorhynchidae sp. of acanthocephalan and 15 *Proleptus obtusus* nematodes

all of which were found inside the stomachs. Given the results of the parasite survey, males and females do not have the same parasites as females have four different species while males only have one. More collections from other areas and times of year are necessary to obtain a better description of the species.

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Chapter 1: INTRODUCTION:

Around 416 million years ago at the end of the Silurian Era, the diversification boom often referred to as the "Age of Fishes" occurred. During this period, the Devonian Era, both freshwater and marine species of fish diversified tremendously. The vast majority of lineages that arose during this epoch are now extinct and largely unknown, or known solely from fossils, yet, the "Age of Fishes" was still an important time as it gave rise to the modern fish we see in marine and freshwater environments today. Fishes of this time had evolved jaws and paired fins which aided in their movements and feeding capabilities (Ebert *et al*., 2013).

1.1 Sawshark Taxonomy

The earliest known fossils of sharks are from the early Devonian period around 409 million years ago (Ebert *et al.,* 2013). According to Benton *et al.,* 2009, the class Chondrichthyes of which the sharks, skates, rays, and chimeras are members, diverged from a common ancestor of bony vertebrates which would stem into Osteichthyes (bony fishes and what would also give rise to tetrapods) around 420 million years ago. These two major groups are the only remaining groups of all those that were present during the Devonian era, with only hagfish and lampreys comprising the once diverse superclass of jawless fishes known as the Agnathans (Romer & Williams 1976). Of the modern sharks, which fall in the subdivision Selachii, there are two major superorders (Galeomorphi and Squalomorphi). The squalomorphs are composed of four orders, of which the Pristiophoriformes were the last to diversify. This order is comprised of eight species of sawshark (Klimley 2013). Seven sawshark species fall into the genus *Pristiophorus* and one species in the genus *Pliotrema*.

1.2 Sawshark Background

Of the eight sawshark species (Fig. 1.1), all eight in the genus *Pristiophorus* have five gill slits. According to the fossil record, sawsharks were once distributed worldwide, however, they are currently only found in small range restricted areas along insular and continental shelves and the upper slopes (Keyes 1979). Aside from *Pliotrema warreni*, the other seven species can be found mainly in the southeast Atlantic Ocean, and both the west Indian and Pacific Oceans (Ebert *et al.,* 2013). Six of the eight species are endemic to the coasts of either southern Africa or Australia. The remaining three species are endemic to the Northern Hemisphere and found in the Northwest Pacific and Atlantic Oceans. Only the dwarf African sawshark *Pristiophorus*

nancyae, has been confirmed to occur from Mozambique to Socotra Islands (Weigmann *et al.*, 2014).

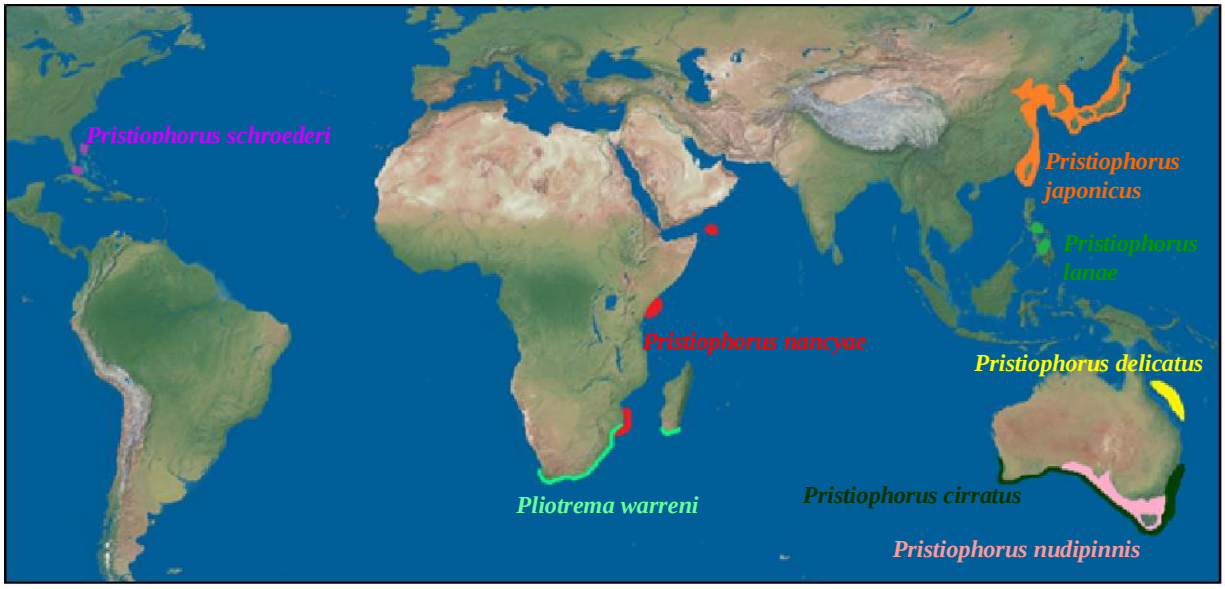

Figure 1.1 Distribution map of the eight extant species of sawsharks. *Pliotrema warreni, Pristiophorus nancyae, Pristiophorus cirratus, Pristiophorus nudipinnis, Pristiophorus delicatus, Pristiophorus japonicus, Pristiophorus lanae, Pristiophorus schroederi* (Photo adapted from http://www.shadedrelief.com/natural3/pages/textures.html).

In the Southern Hemisphere, both the sixgill sawshark *Pliotrema warreni* and the African dwarf sawshark *Pristiophorus nancyae* are endemic to the coastal offshore waters off the coasts of South Africa, Mozambique, and Madagascar (Fig. 1.1). Since *P. warreni* is the subject species of this paper, it will be discussed separately and in more detail, while the other seven species of sawsharks will be discussed in this section.

The other endemic southern African sawshark, *Pristiophorus nancyae* is even lesser known than *P. warreni*. The main occurrence area and the region from which *P. nancyae* was first obtained and described is the western Indian Ocean around the Mozambique coast. Recent reports also indicate that its distribution may also encompass the Kenyan coast as well as the waters around the Socotra Islands (Weigmann *et al.*, 2014). There are unconfirmed reports of this species off the Somalian coast and in the Arabian Sea off Pakistan and speculation that these may be of a separate and potentially undescribed sawshark (Ebert & Cailliet 2011). *Pristiophorus nancyae* occupies a deeper habitat than *P. warreni* being found on the continental slope between depths of 286 and 500 m (Ebert *et al.*, 2013).

Three other species of sawsharks occur in the Southern Hemisphere, all of which have distributions restricted to the coastal offshore areas around Australia. Two of these three species have overlapping distributions (Fig. 1.1). The longnose sawshark, *Pristiophorus cirratus*, has the most extensive distribution along the southern coast of Australia. Much of the distribution of *P. cirratus* is overlapped by that of the shortnose sawshark, *Pristiophorus nudipinnis*. *Pristiophorus cirratus* can be found typically on or around sandy and/or gravel bottomed areas on the upper slope of the continental slope at depths anywhere from 40 to 630 m. Part of its shallow depth range is attributed to its intermittent meanders into coastal inshore areas like estuaries and bays (Ebert *et al.*, 2013).

The second species in this same overlapping distribution is *Pristiophorus nudipinnis* which is found on the bottom of the continental shelf. It can be found at inshore depths of 110 m to possibly depths as deep as 165 m (Ebert *et al.*, 2013).

The third Australian species has a restricted range which does not overlap with the distribution of any of the other Australian sawshark species. The tropical sawshark, *Pristiophorus delicatus*, is the only of the three Australian species which occurs on the northeastern coast of the continent around the Queensland area. This Australian species also favors the environment along the continental slope found between 245 and 405 m deep. *Pristiophorus delicatus* seems to favor not only a different region to the other two Australian sawshark species, but also a more intermediate depth range (Ebert *et al.*, 2013).

The three remaining species of sawshark are endemic to the Northern Hemisphere. Two of these three can be found in the Pacific Ocean off the coasts of Asian nations. The Japanese sawshark, *Pristiophorus japonicus*, has the most expansive distribution of the three Northern Hemisphere sawsharks. This species can be found throughout the northwest Pacific Ocean in the coastal offshore waters around Japan, Taiwan, Korea, and the northern border of China. *Pristiophorus japonicus* prefer the muddy and sandy bottoms of the continental shelf in a relatively deep range 50 to 800 m (Ebert *et al.*, 2013). The second Asian species of sawshark is the Philippine or Lana's sawshark, *Pristiophorus lanae* which has a small distribution encompassing the Philippine Islands, Apo Island and southern Luzon. *Pristiophorus lanae* tends to stick to the upper slopes of the continental shelf along the bottom at depths of 229 to 593 m (Ebert *et al.*, 2013). The Philippine or Lana's sawshark was described by Dr. David Ebert who discovered it in the fish collection in the ichthyology department at the California Academy of Sciences, and named it after his niece (Ebert & Wilms 2013).

The final species is the Bahamas sawshark, *Pristiophorus schroederi*. This species is the only sawshark known to occur in the Atlantic. *Pristiophorus schroederi*, much like *Pristiophorus lanae*, has a small distribution in the northwest Atlantic Ocean in the offshore waters amid Florida, the Bahamas, and Cuba. This species most commonly occurs along the insular and continental slopes near the sea floor at the deepest depth range of all nine sawshark species between 438 and 952 m (Ebert *et al.*, 2013).

1.3 Sawshark Biology

Very little is known of the biology and behavior of elasmobranchs in the order Pristiophoriformes. On the whole, the order is known to be yolk-sac viviparous with their young gaining all vital nourishment from a yolk sac held inside the mother. Sawsharks tend to be quite small with the biggest on record being only 153 cm in length. They can also be distinguished from their sawfish cousins by the presence of barbels on their rostrum, alternating small and large rostral teeth, and gills on their sides as opposed to their ventral surface. Due to the location of the gill openings and spiracles, this allows them to feed and spend much of their time on the sea floor. A final feature possessed by this order is ampullae of Lorenzini on the underside of their rostrum which they use to scan the bottom for prey which they then stun with their saw-like rostrum (Ebert *et al*., 2013).

1.3.1 African dwarf sawshark, *Pristiophorus nancyae*

The first little known species, *P. nancyae*, was described by Ebert & Cailliet, (2011) who found a distinguishing characteristic of the species to be two rows each with four or five obviously sizable pits on the underside of the rostrum anterior to the barbels (Fig. 1.2). Some of their other unique features include the positioning of their barbels much closer to their mouths than to the tip of their rostrum and conspicuous ridges at the base of the largest rostral teeth. *Pristiophorus nancyae* are homogenously brown on their dorsal side, white on their ventral side, and have stripes of dark brown along the center and edges of an otherwise light rostrum (Ebert & Cailliet 2011). As far as the behavior and biology of this species are concerned, very little is known. This species is a small sawshark. The maximum total length of 62 cm was that of an adult male. The size at birth for *P. nancyae* is not known. Males mature at 45 cm and reach adulthood at between 52 and 62 cm, whereas females mature around 57 cm (Ebert & Cailliet 2011). *P. nancyae* are assumed to be yolk-sac viviparous (Ebert *et al*., 2013). They are believed to prey on solely small crustaceans (Ebert & Cailliet 2011).

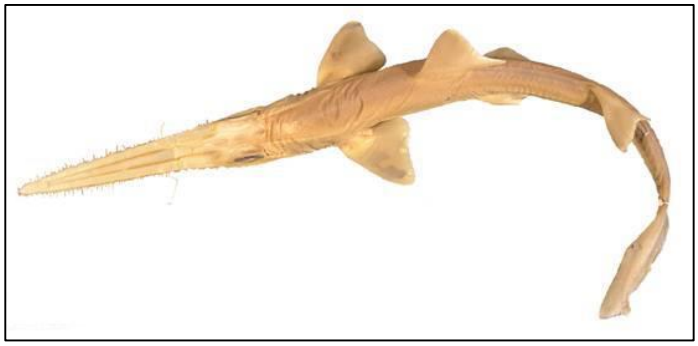

Figure 1.2 Photograph of *Pristiophorus nancyae*, the African dwarf sawshark, from Ebert's original species account (Ebert & Cailliet, 2011).

1.3.2 Longnose or common sawshark, *Pristiophorus cirratus*

The longnose or common sawshark, *Pristiophorus cirratus*, is one of the largest in the order. They have quite long rostrums which are also narrow compared to their stocky body (Fig. 1.3). The barbels are located closer to the tip of the rostrum than to the mouth. These sharks tend to be coloured a yellowish to gray-brown, mottled with stripes or spots and with a pale white underbelly. The rostrum can be identified by the presence of dark brown lines along the edges and down the middle. Black margins delineate the 19-21 large teeth along the rostrum. Juveniles can also be differentiated from adults by the existence of two to three small rostral teeth amid each larger one. Their birth size is between 31-38 cm and males reach maturity around 97 cm while females mature around 107-113 cm. The maximum recorded length of this species stands at 149 cm. Despite this species being more extensively studied than most sawshark species, little is known of their general behavior aside from the fact that they commonly occur in feeding aggregations or schools throughout their range. *Pristiophorus cirratus* are also yolk-sac viviparous and gives birth to anywhere from 6 to 19 pups during alternating winters. These sawsharks feed on crustaceans and relatively small fish (Ebert *et al.*, 2013).

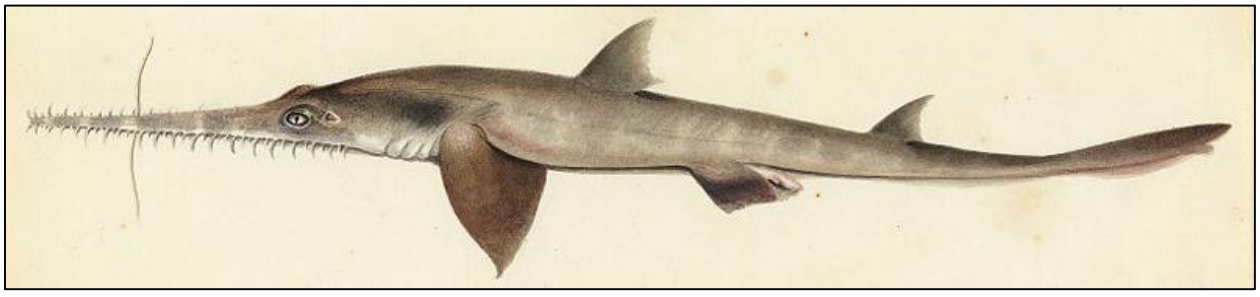

Figure 1.3 Illustration of *Pristiophorus cirratus*, the longnose or common sawshark (William Buelow Gould, 1832).

1.3.3 Shortnose or Southern sawshark, *Pristiophorus nudipinnis*

The shortnose or Southern sawshark are also a stout relatively large species with distinctly shorter and wider rostrums. The barbels are closer to the mouth than the tip of the rostrum with more of the 17-19 total rostral teeth before the barbels than there are after them (Fig. 1.4). Juveniles of this species are noted to have usually only one smaller rostral tooth between each larger one. These sawsharks are evenly gray over their entire dorsal surface with a white underbelly. The rostrums have dark stripes along the middle and edges just as the aforementioned species. At birth, they are 25 to 32 cm long. Males reach sexual maturity around 107 cm and females around 124 cm. The largest recorded individual of this species is a female of 124 cm. Much like other species of sawsharks, they use their ampullae of Lorenzini on the underside of their rostrum to detect and snuff out prey beneath the sand. Again, they are another yolk-sac viviparous species, giving birth to between 7 and 14 pups, though the average litter size is 11, on a biennial basis. *P. nudipinnis* are reported to reach a maximum age of 9 years (Ebert *et al*., 2013).

Figure 1.4 Photograph of *Pristiophorus nudipinnis*, the shortnose or Southern sawshark (Dianne J. Bray, 2011). http://www.fishesofaustralia.net.au/home/species/3524

1.3.4 Tropical sawshark, *Pristiophorus delicatus*

This Australian species are the smallest of the four with a very long, straight-edged rostrum attached to an also very narrow body. Its barbels are located almost equidistant from both the mouth and the rostral tip (Fig. 1.5). Juveniles typically have a few smaller teeth between each of the larger rostral teeth. These sawsharks are usually a yellowish-brown colour on their dorsal surface while also having a white underbelly. Length at birth is not known for this species, though males and females are purported to reach sexual maturity at around 62 cm in length. The maximum recorded individual is 85 cm. Their behavior on the whole is largely

unknown, as is their reproductive characteristics aside from yolk-sac viviparity (Ebert *et al.*, 2013).

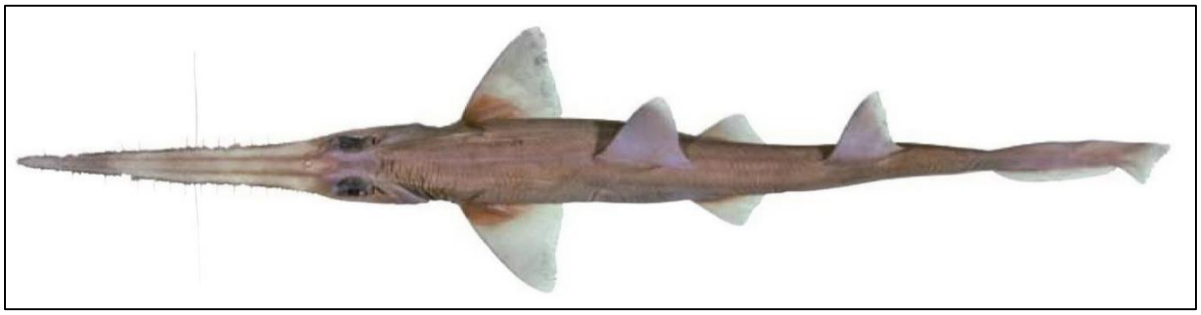

Figure 1.5 Photograph of female holotype of *Pristiophorus delicatus*, the tropical sawshark (Dianne J. Bray, 2011). http://www.fishesofaustralia.net.au/home/species/2611

1.3.5 Japanese sawshark, *Pristiophorus japonicus*

The Japanese sawshark is the largest of all the sawsharks and tends to be quite hefty as well. Their rostrums are about average length compared across the other species in the order and also fairly narrow. The barbels are located closer to the mouth than the tip of the rostrum which has somewhere between 23-43 teeth, depending on size (Fig. 1.6). Juveniles, again, typically possess one or two teeth of a smaller size between the larger rostral teeth. They are either uniformly brown or a red-brown in color on their dorsal side with a uniformly white underbelly and prominent dark brown lines down the center and edges of the rostrum. Birth size is reported at 30 cm for this species with males reported to mature at 107 cm and females at 103 cm. The maximum length range for this species is 136-153 cm. *P. japonicus* are yolk-sac viviparous like all the other species giving birth to about 12 pups in each litter. They use the electroreceptors on their rostrums and their barbels to locate and stun small benthic fish and crustaceans (Ebert *et al.*, 2013).

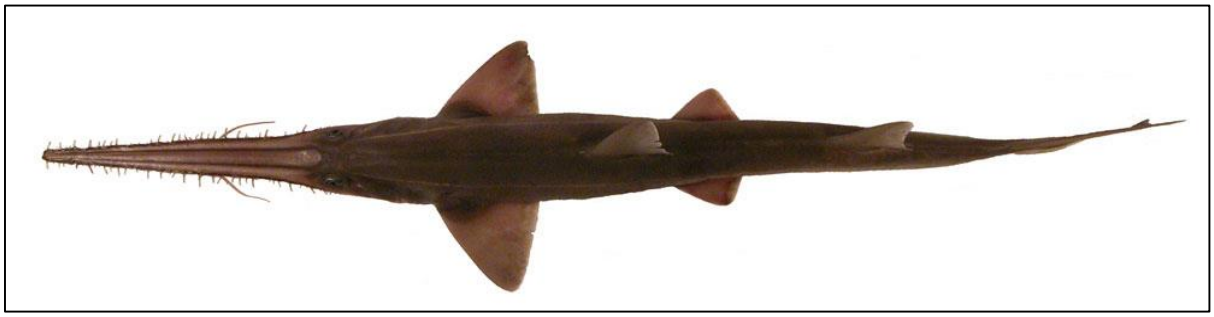

Figure 1.6 Photograph of *Pristiophorus japonicus*, the Japanese sawshark. http://homepage2.nifty.com/megalodon/tokyoprist.html

1.3.6 Philippine or Lana's sawshark, *Pristiophorus lanae*

The Philippine or Lana's sawshark are a relatively small sawshark with short, skinny bodies attached to very extensive, thin rostrums with a uniquely vague concavity between the barbels and the nostrils (Fig. 1.7). That said, the barbels are located closer to the mouth then the rostral tip. Adult rostral tooth number are not known, however, juveniles are reported to have about 21 large rostral teeth with two or three smaller teeth in between. These sawsharks are colored a dark brown over their dorsal surface and also white on their ventral surface (Ebert *et al.*, 2013). As for the rest of the biology and behavior of this species, no literature is currently available. From the few type species analyzed by Ebert and Wilms, the largest was found to be a female of 83 cm and the smallest was the holotype females shown in Figure 1.7 below at 39.6 cm. No males have been analyzed to date. The same Ebert and Wilms study from 2013 also shows that one x-ray of the stomach from the holotype specimen shows the vertebrae of some species of bony fish.

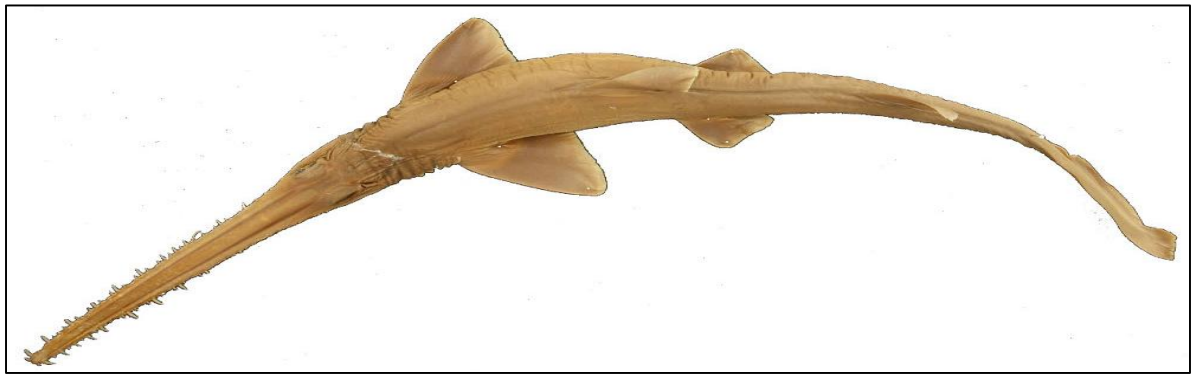

Figure 1.7 Photograph of *Pristiophorus lanae*, the Philippine or Lana's sawshark, holotype from Ebert's original species account (Ebert & Wilms, 2013).

1.3.7 Bahama's sawshark, *Pristiophorus schroederi*

This Atlantic sawshark species is a relatively thin sawshark with long, slender rostrum that possess concave edges prior to the barbels (Fig. 1.8). Those barbels are located equidistant between the mouth and rostral tip. Adults have 23 rostral teeth and juveniles possess typically one smaller tooth between each large one. These individuals tend to be colored uniformly gray on their dorsal surface with a white underbelly. They also have dark brown stripes down the center and edges of their rostrums. The maximum recorded length is 81 cm. Nothing else is known of their behavior or biology (Ebert *et al.*, 2013; Springer & Bullis, 1960).

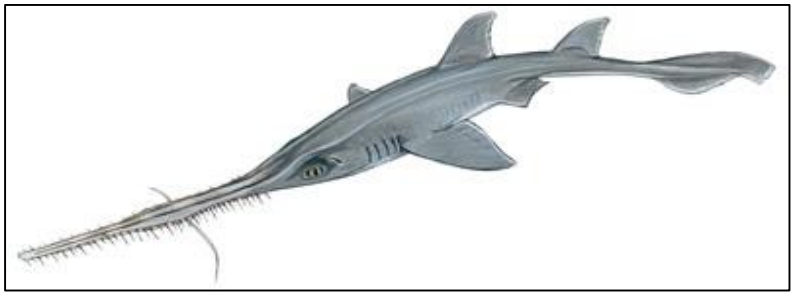

Figure 1.8 Computer generated illustration of *Pristiophorus schroederi*, the Bahama's sawshark (Ebert *et al.*, 2013).

1.4 Sawshark Status and Management

The stock and management status of each of these eight species depends to a large extent on how much their ranges overlap with trawl fisheries, and how well those fisheries are managed. *Pristiophorus nancyae* are listed by the IUCN Red List as a 'Not Evaluated' species due to their recent 2011 discovery. However, given their range, it is possible that they could be a commonly discarded bycatch species in South African and Mozambican deepwater trawl fisheries (Ebert *et al.*, 2013).

Pristiophorus cirratus were assessed by the IUCN Red List as a species of 'Least Concern' due to their relatively large abundance which have been recently surveyed by the Australian government. This shark species is used for food and the current fishery bycatch rates are stable (Ebert *et al.*, 2013). The Australian sawshark, *Pristiophorus nudipinnis*, have been assessed by the IUCN Red List as 'Least Concern'. Population surveys have indicated that it is fairly common throughout its known distribution which explains their current IUCN placement. This species is also utilized for food and subject to bycatch of bottom trawlers and gillnets, though they are subject to similar governmental management strategies as apply to *P. cirratus* in order to monitor their catch rates throughout their range and aim to conserve the population (Walker 2003). *Pristiophorus delicatus*, the remaining Australian sawshark which occupies a separate region of the Australian coast, has been ranked as a 'Least Concern' species according to the IUCN. The reason being low to negligible fishing pressures present over their distribution as well as the lack of fishing effort to the same depth of which this species occupies (Rigby & Heupel 2015).

As it pertains to *Pristiophorus japonicus*, the IUCN lists them as a 'Data Deficient' species. However, regardless of a lack of data about them, they are a bycatch species of a number of Japanese fisheries and their meat is sold as food throughout Japan. Reports indicate

that they are sparse throughout their distribution range which could indicate that their numbers have been substantially depleted (Ebert *et al*., 2013). The other species, *Pristiophorus lanae*, is the most recently discovered and named species. Therefore, its current status on the IUCN Red List stands as 'Not Evaluated'. More knowledge and information about its basic biology and behavior need to be acquired before it can be given a thorough and proper rating (Ebert *et al*., 2013).

Pristiophorus schroederi, are listed by the IUCN as a 'Data Deficient' species. The justification behind their deficient data rating is a concurrent lack of information due to the lack of fishing effort over the depth range occupied by this species around the Bahamas as this species occupies the deepest depth range of all eight species of sawshark (Ebert *et al*., 2013).

1.5 *Pliotrema warreni* Biology

The key difference between the genera is that *Pliotrema* have six gill slits whereas *Pristiophorus* have five. The sixgill sawshark, *P. warreni*, are a benthic and epibenthic species which occur in the southeast Atlantic Ocean and southwest Indian Ocean on the continental shelf and upper slope around South Africa and Madagascar between 37-500 m with adults believed to inhabit deeper areas than pups (Ebert *et al*., 2013). They are one of the 29 southern African endemic shark species. *P. warreni* feed on small fish, crustaceans, and squid (Compagno, 1999; Ebert & van Hees 2015).

The biology, ecology, and behavior of this species are not well known, hence the purpose of this study. Yolk-sac viviparity is similar to viviparity in that there is internal fertilization and live birth, but in this case there is no placenta and the young are nourished from a yolk sac inside the mother (Fowler 2004). Pups are 35-37 cm long at birth and females may have 5-17 pups annually. Males reach sexual maturity at around 83 cm and females around 110 cm. The largest male of the species is recorded at 112 cm and the largest female is recorded at 136 cm (Fowler 2004; Ebert *et al.*, 2013).

Figure 1.9 Map of southern Africa and Madagascar showing the predicted range of *Pliotrema warreni*.

1.6 *Pliotrema warreni* Status

There are little to no conservation or management practices in place for this species. The unique saw-like projections on the extended rostrum of this shark are a disadvantage if they get tangled in nets. Humber *et al*., (2008) reported that from November 2006 – October 2007 0.6% of the recorded catch from the Andavadoaka region of Madagascar was *P. warreni*. Some predictions claim that the rate by which they are discarded as bycatch, combined with their limited range, may very well lead to a collapse of this species (Ebert *et al*., 2013). It is for this reason that the International Union for Conservation of Nature and Natural Resources (IUCN) has listed *P. warreni* as 'Near Threatened' (Fowler 2004).

1.7 Similar Shark Parasites

To date no research has been conducted on parasite species associated with *P. warreni* in South Africa. Some studies have examined the parasites of other sawshark species, namely *Pristiophorus cirratus*, as well as other endemic shark species off South Africa. Previous studies conducted on *P. cirratus* reveal five species of parasites. *Pristiophorus cirratus* was found to have three cestode species (*Flexibothrium ruhnkei*, *Cardiobothrium beveridgei*, and *Bibursibothrium gouldeni*) in the spiral intestine. It also had the myxozoan *Chloromyxum pristiophori* in the gall bladder and the trematode *Otodistomum pristiophori* in the abdominal cavity (Woolcock 1935 & 1936; McKenzie & Caira 1998) (Table 1.1).

In a study comparing three species of South African endemic catsharks (dark shyshark, *Haploblepharus pictus*, puffadder shyshark, *Haploblepharus edwardsii*, pyjama shark, *Poroderma africanum*), they hosted three blood parasites (Yeld 2009). Yeld (2009) also found

one species each of leech (*Stibarobdella macrothela*), copepod (*Perissopus oblongus*), isopod (*Gnathia pantherina*), trematode (*Probolitrema capense*), and a nematode (*Proleptus obtusus*) were found (Table 1.2). Another study (Morris 2015) examined at two sandshark species, the lesser guitarfish or lesser sandshark, *Rhinobatos annulatus,* and the bluntnose guitarfish, *Rhinobatos blochii.* Morris (2015) found a number of parasites other than the families found in this study, though one species of isopod, one species of copepod, two species of cestodes and two species of nematodes were also recovered (Table 1.2). Of the aforementioned studies, the parasites they found which could be used to compare against the parasites found in this study are the isopod, *Gnathia pantherina*, found in the gills, the nematode, *Proleptus obtusus* found in the stomach, and the nematode, *Ascaris* sp., found in the kidney (Yeld 2009, Morris 2015). The percent prevalence for *Proleptus obtusus* for Yeld (2009) was 100% for all three shark species surveyed while Morris (2015) found 31.6% prevalence in *Rhinobatos annulatus* and 29.4% in *Rhinobatos blochii* and the nematode *Ascaris* sp. to be at 11.8% prevalence.

Numerous species of parasite have been found in three species of shysharks endemic to South Africa (dark shyshark, *Haploblepharus pictus*, puffadder shyshark, *Haploblepharus edwardsii* and pyjama shark, *Poroderma africanum*) (Yeld & Smit, 2006; Yeld 2009). Two of these species of shysharks, *Haploblepharus edwardsii* and *Poroderma africanum*, occur in the same range distribution as *Pliotrema warreni*. A number of tetraphyllidean tapeworms have also been documented in a related sawshark species, the longnose sawshark, *Pristiophorus cirratus* (McKenzie & Caira 1998) from Australia. A myxozoan and trematode were also found in *P. cirratus* (Woolcock 1935; 1936). Morris (2015) documented a myriad of parasitic phyla found in two species of guitarfish endemic to the South African and Namibian coasts and occupy a similar niche to *P. warreni*. The only documented parasite from *P. warreni* is copepod *Perissopus oblongatus* (Oldwage & Smale 1993) (Table 1.2).

Table1.1 Parasite species described from sawshark species detailing host species, host distribution, parasite location and paper referenced.

Parasite Species	Parasite Taxon	Parasite Location	Reference
***Pristiophorus cirratus* – Southern Australia**			
Flexibothrium ruhnkei	Cestode	Spiral Intestine	McKenzie & Caira 1998
Cardiobothrium beveridgei	Cestode	Spiral Intestine	McKenzie & Caira 1998
Bibursibothrium gouldeni	Cestode	Spiral Intestine	McKenzie & Caira 1998
Chloromyxum pristiophori	Myxozoan	Gall Bladder	Woolcock 1936
Otodistomum pristiophori	Trematode	Abdominal Cavity	Woolcock 1935
***Pliotrema warreni*- Southern Africa**			
Perissopus oblongatus	Copepode	Petoral fin	Oldewage & Smale 1993

Table 1.2 Parasite species described from South African endemic elasmobranch species which occupy similar ecological niches detailing host species, host distribution, parasite location and paper referenced.

Parasite Species	Parasite Taxon	Parasite Location	Reference
***Haploblepharus pictus* – Western Coast of South Africa**			
***Haploblepharus edwardsii* – South and Eastern Coasts of South Africa**			
***Poroderma africanum* – Western, South, and Eastern Coasts of South Africa**			
Haemogregarina sp. A	Haemogregarine	Blood	Yeld 2009
Haemogregarina sp. B	Haemogregarine	Blood	Yeld 2009
Trypanosoma sp. A	Trypanosome	Blood	Yeld 2009
Trypanosoma haploblephari	Trypanosome	Blood	Yeld & Smit 2006
Stibarobdella macrothela	Leech	Skin	Yeld 2009
Perissopus oblongus	Copepod	Skin	Yeld 2009
Gnathia pantherina	Isopod	Gills and Skin	Yeld 2009
Probolitrema capense	Trematode	Body Cavity	Yeld 2009
Proleptus obtusus	Nematode	Stomach	Yeld 2009
***Rhinobatos annulatus* – Namibian and South African Coasts**			
Echinobothrium dougbermani	Cestode	Spiral Valve	Caira *et al.*, 2013
Trichodina rhinobatae	Ciliophora	Urogenital Tract	Van As & Basson 1996
Gnathia pantherina	Isopod	Gills	Hayes *et al.*, 2007
Pseudoleptobothrium christisoni	Monogenean	Dermal Denticles	Vaughan & Chisholm 2011
Neoheterocotyle hargis	Monogenean	Gills	Vaughan & Chisholm 2010
Proleptus obtusus	Nematode	Stomach	Morris 2015
Ascaris sp.	Nematode	Kidney	Morris 2015
Trilocularia sp.	Cestode	Spiral Valve	Morris 2015
Clavelotti sp.	Copepod	Gill Arch	Morris 2015
***Rhinobatos blochii* – Namibian and Western South African Coasts**			
Proleptus obtusus	Nematode	Stomach	Morris 2015
Ascaris sp.	Nematode	Kidney	Morris 2015
Clavelottis sp.	Copepod	Gill Arch	Morris 2015

1.8 Aims/Objectives

The aims of this project are largely two-fold. The largest portion of this study is dedicated to the detailed biological assessment of the sixgill sawshark, *Pliotrema warreni*. This assessment analyzed morphology, reproductive biology, and feeding ecology. The second portion of this study is aimed at providing an assessment of macroparasite load.

Chapter 2: MATERIALS/METHODS:

2.1 Study Site

Thirty specimens of *Pliotrema warreni* were caught by trawling near Bird Island in Algoa Bay on the Eastern Cape of South Africa between 50 and 120 m depth during a trawl survey in May 2015. These were immediately put on ice and later frozen and brought to the University of Cape Town where they were thawed for dissection and analysis. Two other specimens were collected by the Department of Agriculture, Forestry and Fisheries in another trawl survey completed in April 2015 at coordinates 34.18°S, 25.44°E outside Sardinia Bay. All samples were frozen onboard and taken to UCT for dissection and analysis.

2.2 Morphometric Dissections and Parasite Collection

All specimens were thawed completely overnight and weighed to the nearest gram (g) on an electronic balance. All length measurements were recorded to the nearest millimeter (mm) using a measuring board or flexible measuring tape (Fig. 2.1). Table 2.1 lists the measurements which were recorded from each shark. Each shark was examined externally for ectoparasites on the skin, gills, mouth, eyes, or nares. Parasites found were counted, removed and placed in individually marked vials of 40% formalin solution. Basic parasite statistics (prevalence, abundance, intensity) were recorded using Bush *et al.* 1997. Three photographs were taken of each shark from a lateral view, aerial view, and from the underside with a Sony Cyber-shot DSC-TX20 16.2 megapixel Exmor R CMOS digital camera with 4x optical zoom. A piece of vertebrae about three inches long was taken from just before the 1st dorsal origin for age determination.

Figure 2.1 Photographs of the dorsal and ventral views of *Pliotrema warreni* following thawing and prior to dissection when all external morphometric measurements were collected.

Each shark was opened using dissecting scissors along the midline of the underside of the body from the cloacal opening to just above the heart taking care not to cut or otherwise damage any of the internal organs (Table 2.1). When retrieving the stomach, the entire digestive tract was removed from the lower esophageal sphincter to the termination of the spiral valve at the cloacal opening. The stomachs were separated from the spiral valve at the pyloric sphincter, weighed, and placed in a jar containing a 30% formalin solution with the corresponding numbered tag for each shark and stored for later dissection for content analysis. All organs aside from the stomach were assessed both visually as well as biopsied and prepared on slides for analysis under a Leica DM750 compound light microscope with 4x-100x optical magnification to determine any potential microscopic parasite presence. All parasites found were photographed with a Canon EOS 360D digital camera with a microscope attachment.

The final stage internal examination consisted of an assessment of the reproductive organs (Table 2.1). For male sharks, the clasper state was determined on a scale of 1-3 with 1 being small and non-articulate and 3 being large and capable of use during copulation. Articulation and sperm presence were recorded. The testes were weighed individually (left and right) and the clasper lengths were measured for both sides. For female sharks, maturity or immaturity was first determined based on the presence of fertilized eggs in the ovaries. If immature females, only the number of eggs greater than 10 mm in diameter and ovary weights were recorded. All specimens were examined for internal and external parasites, no blood samples

were used for determination of parasite presence due to the prolonged exposure to harsh freezer conditions between capture and examination. The gonadosomatic index (GSI) was also determined using the equation [gonad mass / total body mass] x 100 (Barber & Blake 2006).

Table 2.1 Summary of all morphometric measurements both internal and external taken from each *Pliotrema warreni* during dissections.

Location	Measurement
External Morphometrics	Total Mass (g)
	Total Length (mm)
	Standard Length (mm)
	Girth (mm) [taken at 1st dorsal origin]
	Rostrum Length (mm) [tip to interoccular termination]
	Number of Rostral Teeth
	Age Sample
Internal Measurements	Liver Color
	Liver Mass (g)
	Heart Mass (g)
	Stomach Mass (g)
	Interoccular - Pre Caudal Length (mm)
	1st Dorsal Origin - Pre Caudal Length (mm)
	1st Dorsal Origin - 2nd Dorsal Origin Length (mm)
	Mouth Width (mm)
Reproductive Measurements (Females)	Maturity or Immaturity
All Females	Number of Eggs > 10 mm
	Ovary Mass (g) [left and right]
Mature Females Only	Oviducal Gland Length (mm) [left and right]
	Oviducal Glad Width (mm) [left and right]
	Widened Uterus (yes or no)
	Uterus Mass (g) [left and right]
Reproductive Measurements (Males)	Clasper State (1, 2, 3)
	Clasper Length (mm) [left and right]
	Clasper Articulation (yes or no)
	Sperm Presence (yes or no)
	Teste Mass (g) [left and right]

2.3 Stomach Content Analysis

Preserved stomachs were removed from formalin and rinsed externally with water before dissection. The solid contents were separated from the liquid content and each were weighed and recorded. The contents were all examined under a Leica EZ4 stereomicroscope with 4.4:1 optical zoom for any distinguishable prey items. Full fish vertebrae were removed, measured, and photographed. Unique prey items (i.e., gelatinous globs, shrimp heads, etc.) were

separated and noted. Solid and liquid contents were searched for parasites, which were preserved in marked vials of 30% formalin solution for further analysis. All parasites found were photographed with a Canon EOS 360D digital camera with a microscope attachment. The number of items which were found in the stomachs were summed and placed into a pie chart based on percent occurrence. The digested material was marked as one unit for each stomach in which it was found.

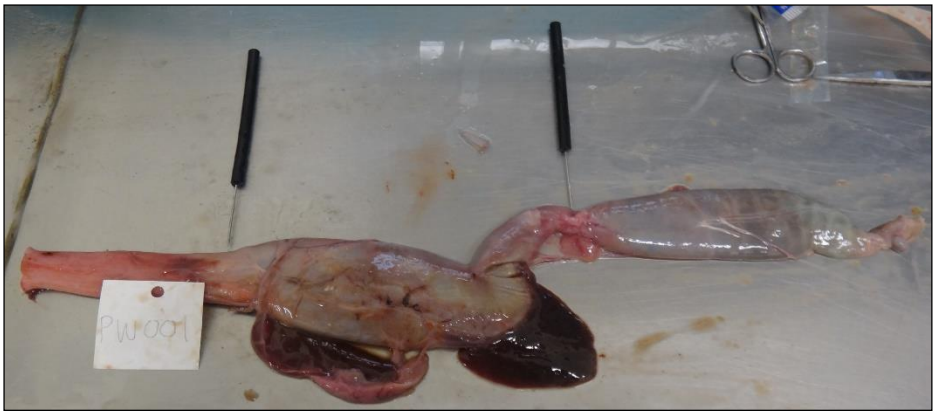

Figure 2.2 Digestive tract of *Pliotrema warreni* with points showing where to separate the stomach from the esophagus (left) and spiral valve (right).

2.4 Vertebral Age Determination

Vertebral samples were boiled individually in water then scrapped clean of all remaining flesh. Each individual vertebra was cut and separated. The neural and haemal arches (transverse processes) were removed and the intervertebral discs were peeled out from the centrum on each side of the vertebra. The vertebra cleaning and remaining procedure for preparing the samples followed the technique outlined in Goosen & Smale (1997) and Cailliet & Goldman (2004). To remove the remaining connective tissue surrounding each centrum, the samples were soaked in 3% sodium hydroxide (NaOH) solution for 3-13 min depending on the size of the vertebra and all remaining connective tissue was scrapped off with a scalpel. Samples were rinsed in distilled water and dried before being set in resin tubes. The resin composed of 100 ml of Polylite clear casting resin and 2.5 ml of PE catalyst andanox KP-9 organic peroxide used as a hardener for the resin setting process. Upon total hardening of the resin tubes, each sample was cut and a 0.5 mm thick slice of the vertebra was taken through the focus of each centrum using a double-bladed rotary diamond saw. These sections were soaked in Alizarin red supernatant in a 1:18 ratio (1ml Alizarin red to 18 ml sodium hydroxide) for two min. Samples were then rinsed in tap water for ten min and placed into a 3% hydrogen peroxide (H_2O_2) bath for one hour. Samples were briefly rinsed in tap water and dried before

being mounted on a slide with DPX mountant medium onto a glass slide. Samples were viewed using a dissecting microscope to count age bands.

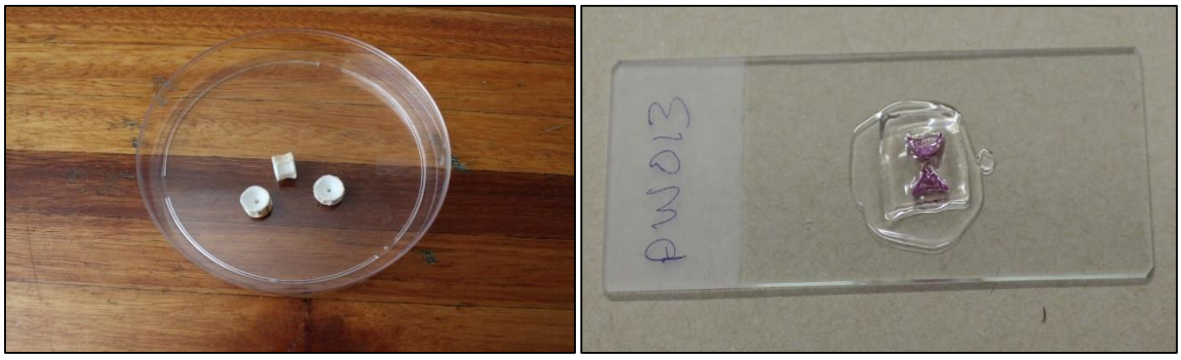

Figure 2.3 Vertebral samples removed from a section of the pre-first dorsal vertebral column of *Pliotrema warreni* following cleaning and drying techniques (left) which were set in polymer resin, cut into sections, stained, and mounted on a slide (right) for age band counting

2.5 Statistical Methods

All morphometric data were compared against total length to describe morphological relations, using the least square regression procedure (Zar 1999). Mann-Whitney U test were used to test for differences in mass, length, rostral length, organ mass, and parasite loads between the sexes. The relationship between the number of rostral teeth and rostral length was tested with the least-squares regression procedure.

Stomach contents were analyzed. Percent frequency of prey items and percent occurrence of prey species were calculated (Bowen 1996). Prey size was correlated with specimen size using the least square regression (Zar 1999). The percent of prevalence of each group of parasite, the parasite intensity and abundance was calculated following Bush *et al*., (1997). Prevalence is the number of host individuals infected with one or more individuals of a given parasite species or group divided by the number of host individuals examined for that parasite species. Intensity is the number of individuals of a given parasite species in a single host. Abundance is the number of individuals of a given parasite species in a measured unit from a host or habitat (area, volume, or weight) (Bush *et al*., 1997). Parasite load was correlated with length using a lease squares regression procedure.

Mann-Whitney U tests were used to test a number of null hypotheses.

1. Males and females have the same average mass.

2. Males and females have the same average length.
3. Morphometric measures and organ masses, as a function of total length or mass, do not vary between sexes.
4. The number of rostral teeth increases with length of the rostrum.
5. Males and females have the same number of parasites.
6. Male and female parasite assemblages do not differ.
7. The intensity of infection by parasites increases with host size.
8. Males and females are the same total length at the same ages.

Chapter 3: RESULTS:

3.1 Morphometrics

Linear equations were used to describe the relationships between total length of *Pliotrema warreni* and other morphometric measurements. Only 31 of the 32 samples were used. One specimen had its rostrum cut off and that fish was used only for the parasite study, stomach content analysis, and the age determination study.

Table 3.1 All equations for the morphometric comparisons to total length compared by sex and by left and right for the sex characteristics of *Pliotrema warreni*.

Correlation	Sex	Equation
ln Total Mass (g) vs. ln Total Length (mm)	Female	ln(TM) = 0.299*ln(TL) + 2.038
	Male	ln(TM) = 0.332*ln(TL) + 1.941
Standard Length (mm) vs. Total Length (mm)	Female	SL = 0.836*TL - 13.292
	Male	SL = 0.864*TL - 35.263
IO-PC Length (mm) vs. Total Length (mm)	Female	IP = 0.606*TL - 43.676
	Male	IP = 0.620*TL - 52.506
DO_1-PC Length (mm) vs. Total Length (mm)	Female	DP = 0.372*TL - 17.366
	Male	DP = 0.403*TL - 39.775
DO_1-DO_2 Length (mm) vs. Total Length (mm)	Female	DD = 0.245*TL - 26.677
	Male	DD = 0.259*TL - 34.535
Mouth Width (mm) vs. Total Length (mm)	Female	MW = 0.040*TL + 4.666
	Male	MW = 0.049*TL - 5.740
Girth (mm) vs. Total Length (mm)	Female	G = 0.243*TL - 24.244
	Male	G = 0.194*TL + 14.522
Rostrum Length (mm) vs. Total Length (mm)	Female	RL = 0.221*TL + 42.514
	Male	RL = 0.235*TL + 28.228
Number of Rostral Teeth vs. Rostrum Length (mm)	Female	RT = -0.302*RL + 255.880
	Male	RT = -0.237*RL + 229.690
Stomach Mass (g) vs. Total Length (mm)	Female	SM = 0.178*TL - 116.080
	Male	SM = 0.115*TL - 60.365
Stomach Mass (g) vs. Total Mass (g)	Female	SM = 0.038*TM - 3.237
	Male	SM = 0.021*TM + 18.413
	Male	
Clasper Length (mm) vs. Total Length (mm)	Left	CL = 0.141*TL - 76.195
	Right	CL = 0.148*TL - 81.857
Teste Mass (g) vs. Total Length (mm)	Left	TeM = 0.025*TL - 16.318
	Right	TeM = 0.025*TL - 17.651
	Both	TeM = 0.051*TL - 33.969
	Female	
Ovary Mass (g) vs. Total Length (mm)	Left	OM = 0.073*TL - 50.476
	Right	OM = 0.056*TL - 35.520

On average, females were about 994 mm long whereas males were 891.82 mm long making females 11.5% longer than males. However, despite females appearing longer than males the results of a Mann-Whitney U test (U-value = 43; $p>0.05$) showed that there is no statistically

significant difference in the total lengths between the sexes. Females on average were 1702 g and males were 1132 g, making females 50.3% heavier than males. The results of the Mann-Whitney U test (U-value = 39; p>0.05) for mass concluded that there is a significant difference in mass between sexes. There is a very strong positive correlation between mass and length denoting that length and mass increase with each other according to the power function (Table 3.1) with a p-value of $4.66x10^{-17}$ for females and $2.22x10^{-4}$ for males (Fig. 3.1). The heavier females could be correlated with the larger total length of females as opposed to males. Males ($R^2 = 0.96$) have a stronger correlation between length and mass than females ($R^2 = 0.98$). The R^2 values for standard length compared to total length for males is 0.99 (p-value = $7.48x10^{-6}$) and 0.99 for females (p-value = $4.66x10^{-17}$) (Fig. 3.2). Standard length was 82.3% (80.7%-83.2%) of the total length for females and 82.4% (81.5%-83.8%) for males.

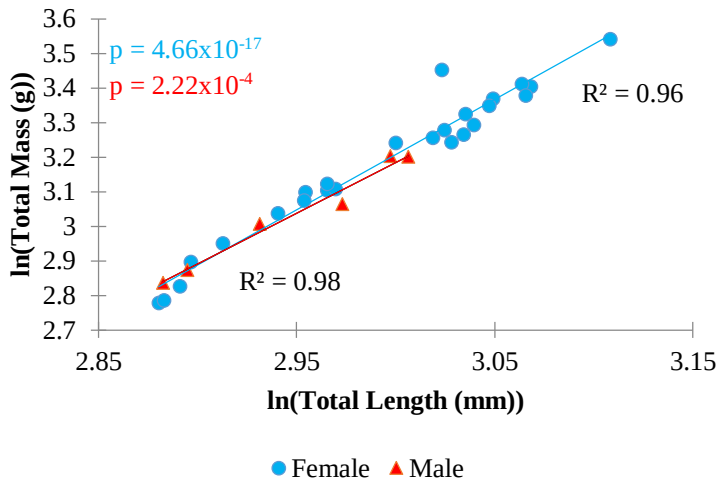

Figure 3.1 The relationship between the ln(mass) and the ln(total length) of *Pliotrema warreni* males and females in southern Africa

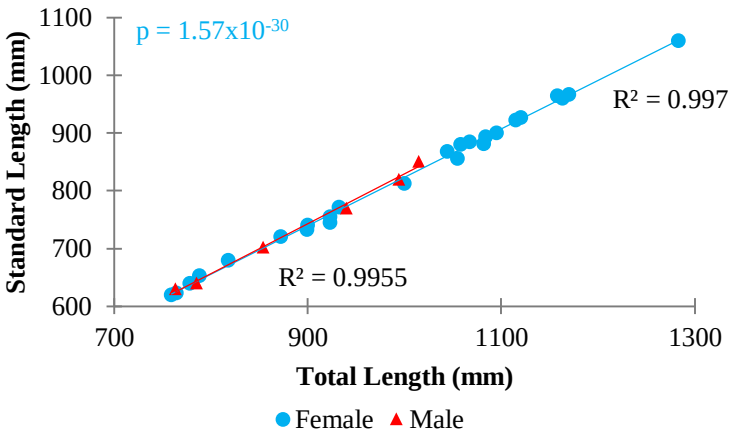

Figure 3.2 The relationship between standard and total lengths of *Pliotrema warreni* for each sex

The interoccular to pre-caudal (IO-PC) lengths compared against total length were also significant (IP = 0.6065*TL – 43.676 females; IP = 0.6205*TL – 52.506 males) (Fig. 3.3). The IO-PC lengths for females were 56.2% of the total length whereas for males they were 56.1%. The relationship for females and males has a strong positive correlation between the first dorsal origin to pre-caudal length (DO_1-PC) vs. total length and the data sets for both sexes fall within the limits of statistical significance (Fig. 3.4). Female DO_1-PC length was 35.5% of the total length and 38.0% for males. The DO_1-DO_2 length was 21.7% of the total length for females and 22.1% for males (Fig. 3.5).

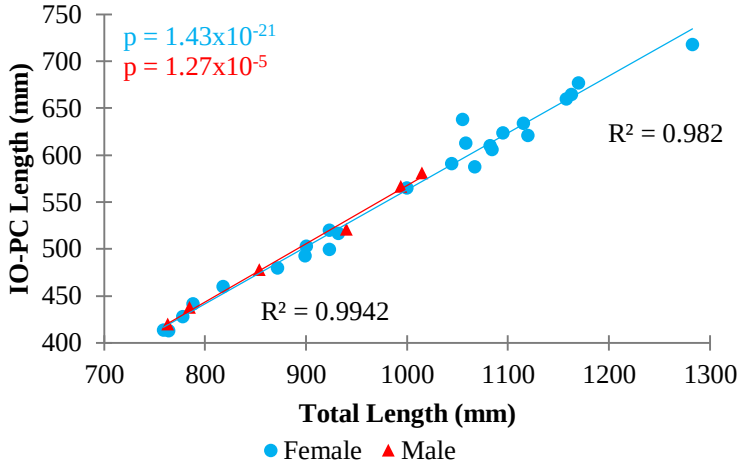

Figure 3.3 The relationship between the interoccular-pre-caudal length and total length of *Pliotrema warreni* by sex

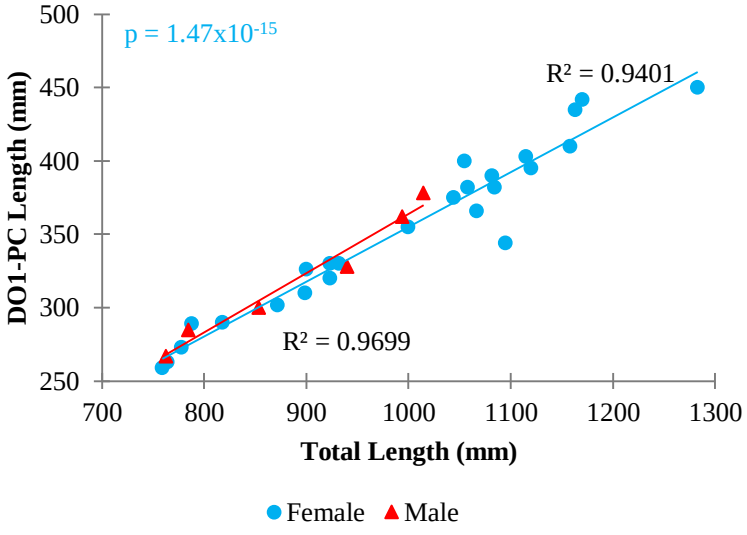

Figure 3.4 The relationship between the first dorsal origin-pre-caudal length and total length of *Pliotrema warreni* by sex

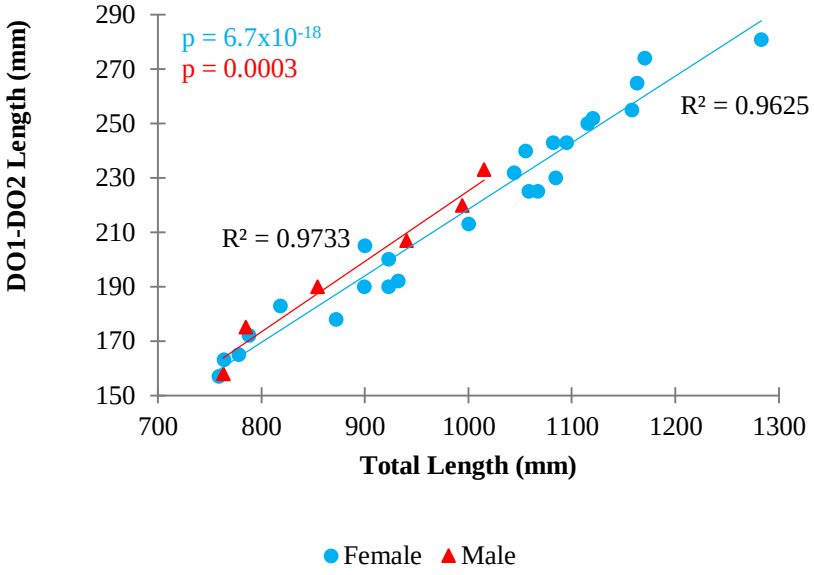

Figure 3.5 The relationship between the first and second dorsal length and total length of *Pliotrema warreni* by sex

The average mouth width for females was 44.9 mm and 36.7 mm for males (Fig. 3.6). The mean girth for females was 217.8 mm while only 178.5 mm for males (Fig. 3.7). The relationship between total length and girth is stronger in males than in females.

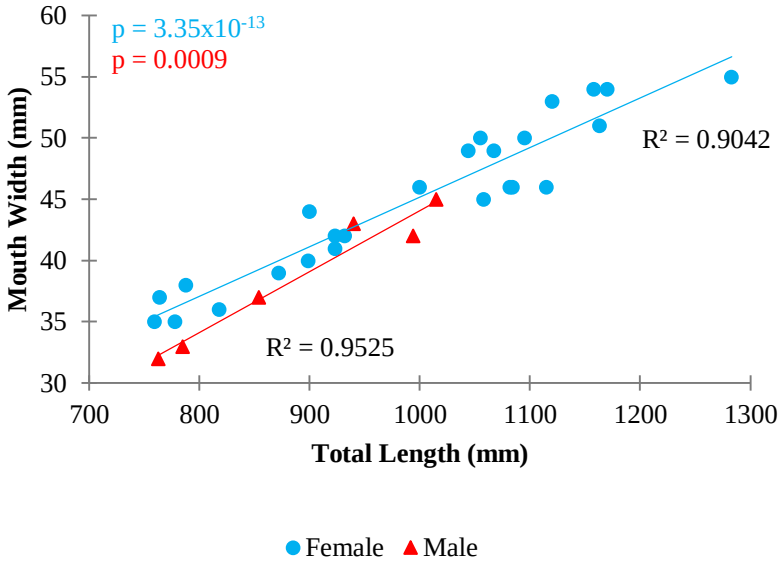

Figure 3.6 The relationship between mouth width and total length of *Pliotrema warreni* by sex

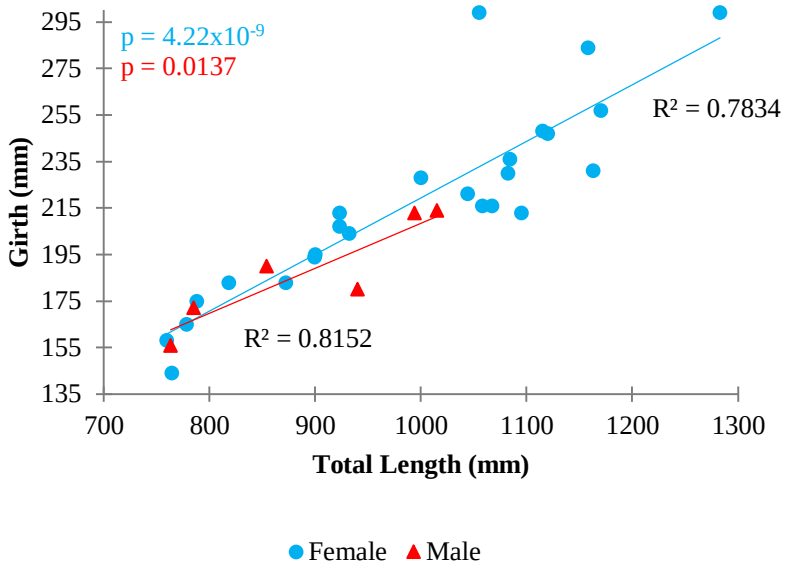

Figure 3.7 The relationship between girth and total length of *Pliotrema warreni* by sex

Rostrum length was also found to be between 24.4%-28.1% of the total length with the average being 26.6% for females and between 26.1%-28.0% of the total length with the average being 26.7% for males (Fig. 3.8). The relationship between rostrum length and the number of rostral teeth was hypothesized to be a positive linear relationship. However, these data indicate that there is a negative linear relationship (Fig. 3.9). The average tooth count was about 176 rostral teeth for females and 173 for males.

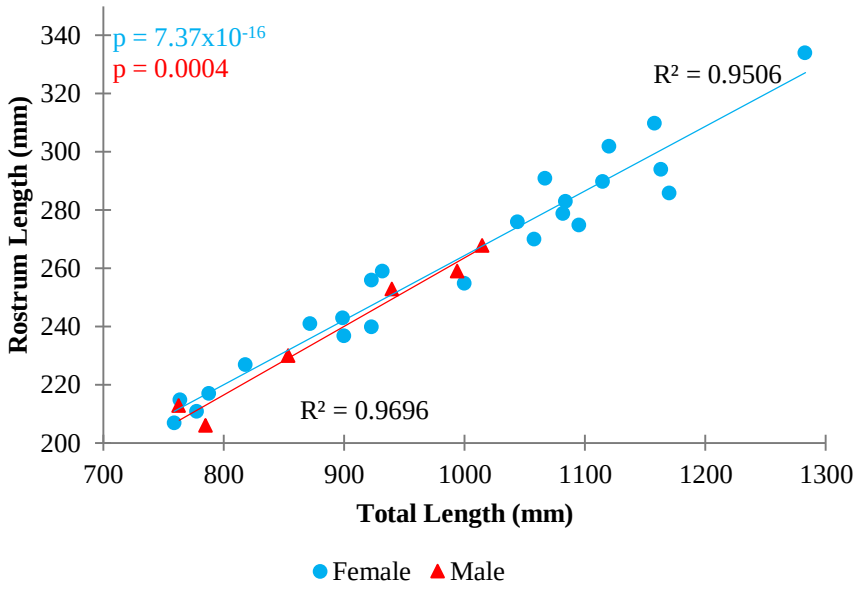

Figure 3.8 The relationship between rostrum length and total length of *Pliotrema warreni* by sex

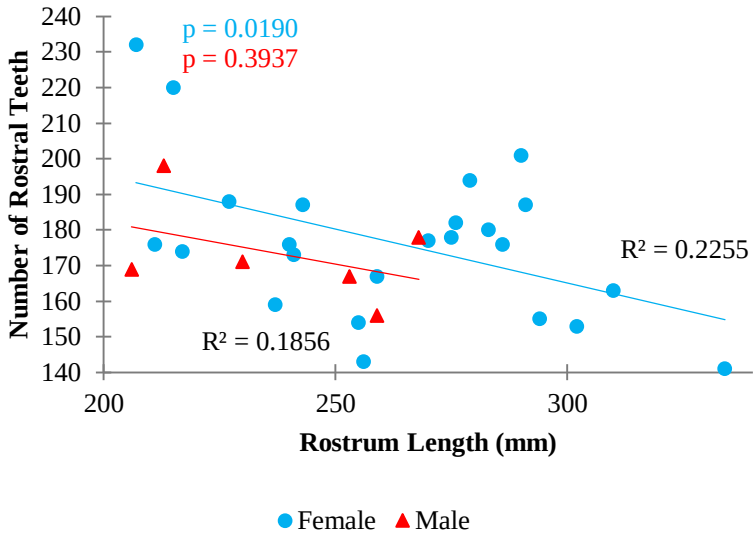

Figure 3.9 The relationship between number of rostral teeth and rostrum length for *Pliotrema warreni* by sex

For the females, the liver was found to be 8.1% of their total body mass while in males it was 5.6% on average (female coefficient of variation = 51.6%; male coefficient of variation = 48.1%). The results of the Mann-Whitney U test (U-value = 41; $p<0.05$) suggest that there is significant difference between average liver mass between sexes. Average female stomach masses were 3.7% of their total body mass while in males it was 3.9%. The Mann-Whitney U test results (U-value = 51; $p>0.05$) conclude there is no significant difference between male and female stomach masses. Female average heart mass to body mass was calculated at 0.08% while males were 0.11%. The conclusion from the results of the Mann-Whitney U test (U-value = 74; $p>0.05$) are that there is no significant difference between heart masses between sexes. In one of the specimens, there was a deep laceration inside the body cavity along the spine and the liver was drastically smaller, even missing a lobe, though the cause remains unknown (Fig. 3.10). The stomach of this individual was also so full that the contents were spilling out of the mouth.

Figure 3.10. Drastically undersized liver of *Pliotrema warreni* from South Africa with only one lobe (107mm) and severe, deep internal puncture and laceration in body cavity of specimen PW024.

All but the smallest two males were sexually mature based on the premise that sperm presence indicated the onset of sexual maturity (Table 3.2). The smallest three were sexually immature based on the premise that clasper articulation and clasper state indicate the onset of sexual maturity. The testes were not visible to the naked eye in the smallest sample.

Table 3.2 All recorded male *Pliotrema warreni* sex statistics showing the maturity state based on clasper data as well as the presence of sperm and mass of the testes.

Total Length (mm)	Clasper State	Clasper Length Right (mm)	Clasper Length Left (mm)	Clasper Articulation	Sperm Presence	Testes Weight Right (g)	Testes Mass Left (g)	Testes Mass Total (g)
940	3	71	68	Yes	Yes	5	8.5	13.5
785	1	39	40	No	Yes	5	5	10
1015	2	56	56	Yes	Yes	9.5	8	17.5
994	2	65	63	Yes	Yes	8.5	9.5	18
854	2	58	57	Yes	Yes	3.5	3.5	7
763	1	16	16	No	No	1	2	3
N/A	1	10	10	No	No	N/A	N/A	N/A

Clasper length appears to reach a peak length before apparently getting smaller as the individual grows larger indicating an interesting negatively allometric relationship between clasper length and total length (Fig. 3.11). The relationship between testis mass and total length is positively correlated with R^2 values of 0.77, 0.79, and 0.84 for the right, left and both testes, respectively (Fig. 3.12). The relationship between testis mass and total length is relatively strong for each individual testis and even stronger still when the testes are combined. The average gonad mass was 11.5 g for males. The GSI for males is 1.02%.

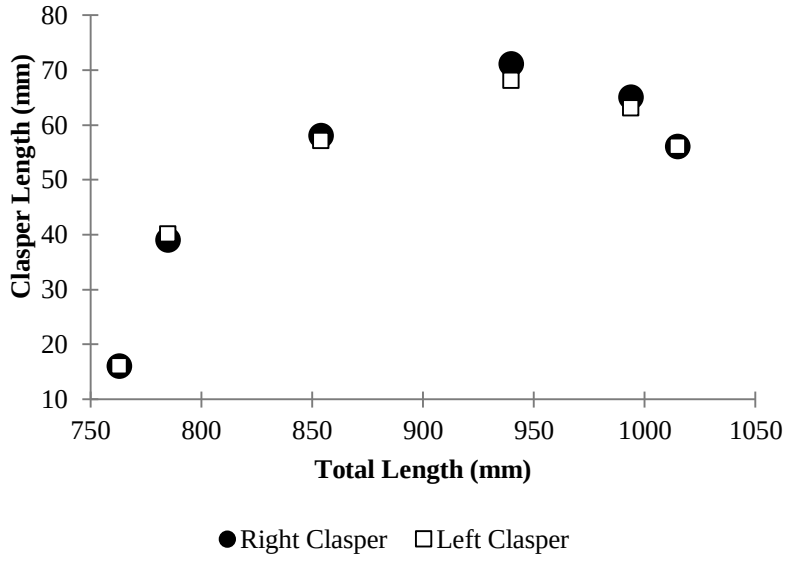

Figure 3.11 A scatterplot of left and right clasper lengths of *Pliotrema warreni* compared to total length.

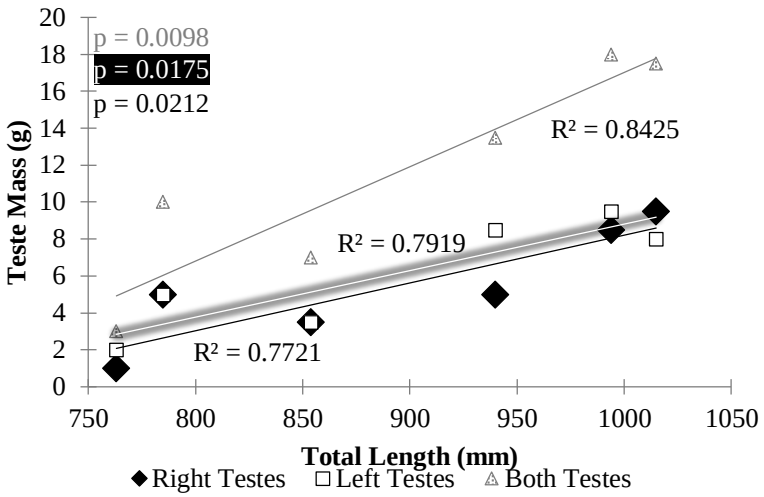

Figure 3.12 The relationship between testis mass for individual and combined testes against total length of *Pliotrema warreni*

Figure 3.13 shows that at 50% maturity, the length of mature females fall within the range 1001-1100 mm and Table 3.3 shows that the females falling within this same range were all mature. The maturity state, number of eggs present in the ovaries which were greater than 10 mm in diameter, and whether or not the uterus had widened in preparation for passage of eggs for development shows that all the females found in the classes 1001-1100, 1101-1200 and 1201-1300 were mature. If the individual was mature then, in all cases, the uterus had widened for the preparation of embryo development and is further supported by the fact that all mature individuals also possess distinctly heavier ovary and uterine masses than immature individuals. In all of the 25 females in the study, only two of them were mature enough to have had fertilized, well-developed eggs present in the ovaries. Both of the individuals which contained these large eggs were over 1000 mm which is well within the range of known female maturity levels. Some of the immature individuals, while they displayed no uterine widening, happened to display distinctly developed ovaries. However, given that there was no discernible uterine widening, the uteri remained as tiny tubules and were, therefore, not weighed.

Table 3.3 Female *Pliotrema warreni* reproductive data collected during dissections denoting maturity state of individuals as determined by uterus state and segregated by size classes.

Total Length (mm)	No. of Individuals	No. of Mature	Number of Eggs > 10mm	No. with Uterus Widened
701-800	4	0	0	0
801-900	4	0	0	0
901-1000	4	1	0	1
1001-1100	7	7	12	7
1101-1200	5	5	0	5
1201-1300	1	1	14	1

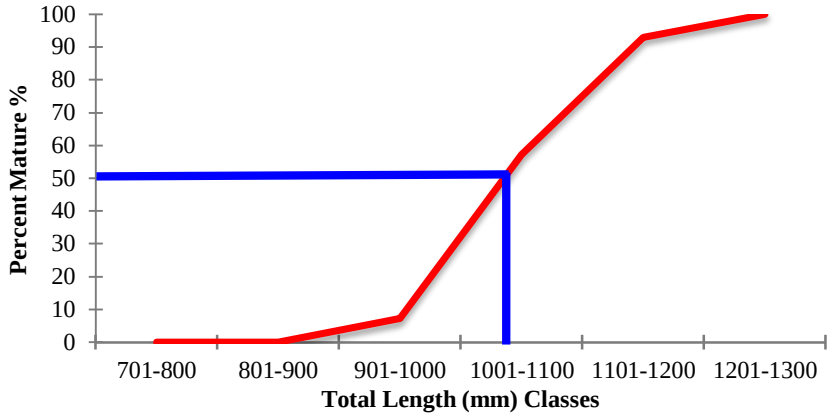

Figure 3.13 Ogive curve of percent maturity of female *Pliotrema warreni* per length class vertical and horizontal line denoting female length at 50% maturity.

There is no relationship between ovary mass and total length (right ovary p-value = 0.54; left ovary p-value = 0.50) (Table 3.4; Fig. 3.14). Uterine mass were only recorded for mature females. The average gonad mass for females was 38.3 g. The average GSI for mature females is 2.25%.

Table 3.4 Female *Pliotrema warreni* reproductive organ statistics with emphasis on ovary and uterine average masses

Total Length (mm)	Average Ovary Mass Total (g)	Average Uterus Mass Total (g)
701-800	8.75	N/A
801-900	7.75	N/A
901-1000	10	2.5
1001-1100	89.57	35.14
1101-1200	17.8	21.1
1201-1300	135.5	34

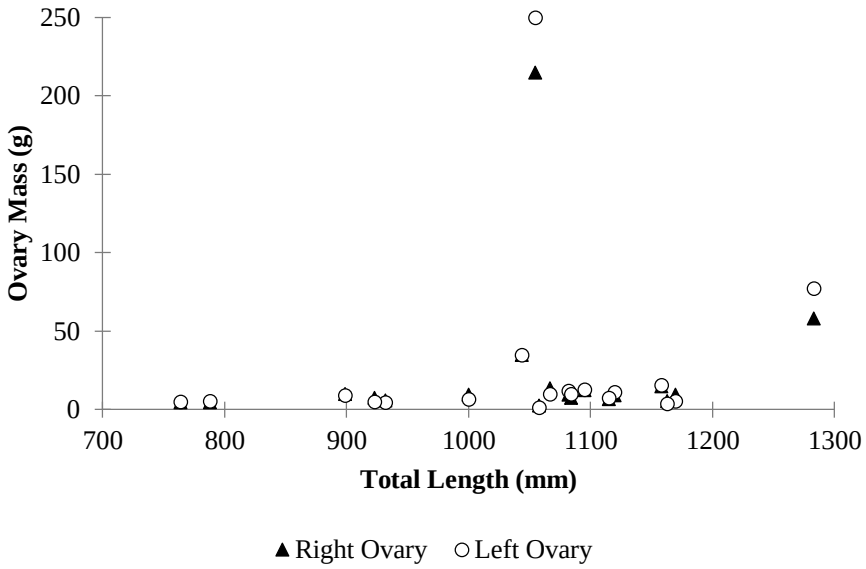

Figure 3.14 A scatterplot of total length and ovary mass showing an insignificant relationship in both ovaries for *Pliotrema warreni*.

Stomach mass was correlated with total length, with a p-value of $9.65x10^{-6}$ (R^2 for females = 0.51; males = 0.21) (Fig. 3.15). Stomach mass also correlated with total mass with a p-value of $7.48x10^{-7}$ (R^2 for females = 0.61; males = 0.10) (Fig. 3.16). Females had an average stomach mass of 61.74 g, while males an average of 38.14 g. Female stomachs comprised 27.6% of the average total body mass and males 29.7%.

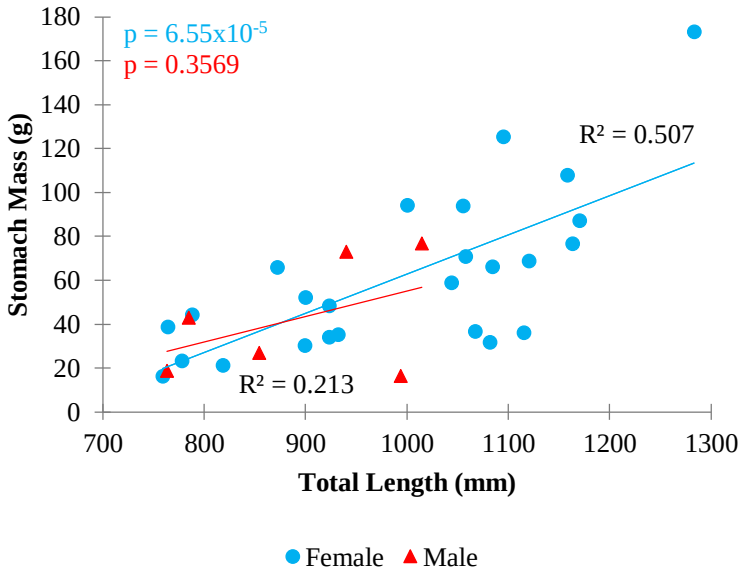

Figure 3.15 The relationship between stomach mass and total length of *Pliotrema warreni* by sex

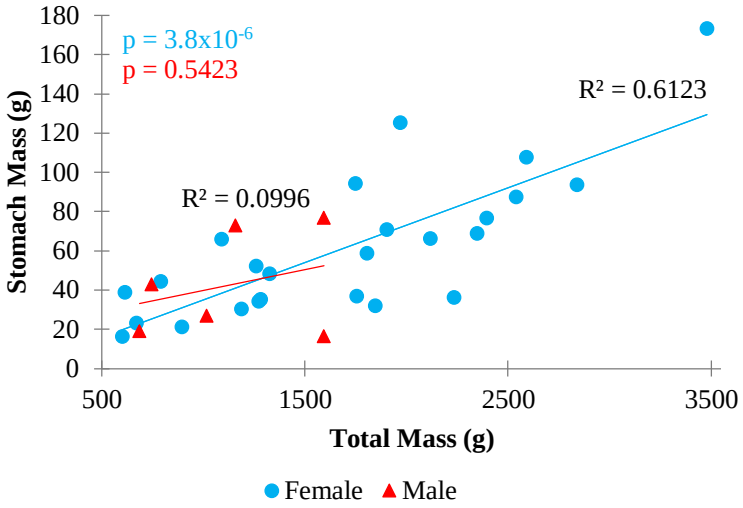

Figure 3.16 The relationship between stomach mass and total mass of *Pliotrema warreni* by sex

3.2 Stomach Content Analysis

A total of six different items were found inside the sample of stomachs which can be grouped into four categories; fish vertebrae, shrimp, digested fish material, and parasites. Of the 32 stomachs which were assessed, seven were devoid of any fish vertebrae but still had digested fish material (Table 3.5).

Table 3.5 List of items found in the stomachs and the number of stomachs of *Pliotrema warreni* which contained each item and the total number of each item which was found

Item	No. of Stomachs	No. of Items
Cape horse mackerel vertebrae	1	1
Redeye round herring vertebrae	24	75
Shrimp	3	9
Digested Fish Material	31	31

The digested fish material was not easily discernible to species level. However, given that the most intact vertebrae were identified as redeye round herring, *Etrumeus whiteheadi*, it was assumed that much of the other digested fish meat material was also redeye round herring. The identification of the relatively intact vertebrae with flesh still on them was made even more difficult due to the heads being highly digested. One of the stomachs was also found to be considerably larger than all of the rest. Inside that stomach was one partly digested Cape horse mackerel, *Trachurus trachurus capensis*, carcass as well as one whole clean vertebrae and three shrimp heads (Fig. 3.8). Of the fish vertebrae and shrimp which were identifiable redeye round

herring comprised 64.96%, shrimp 7.69%, and Cape horse mackerel 0.85% of the diet. The digested material was assumed to come from the vertebrae present.

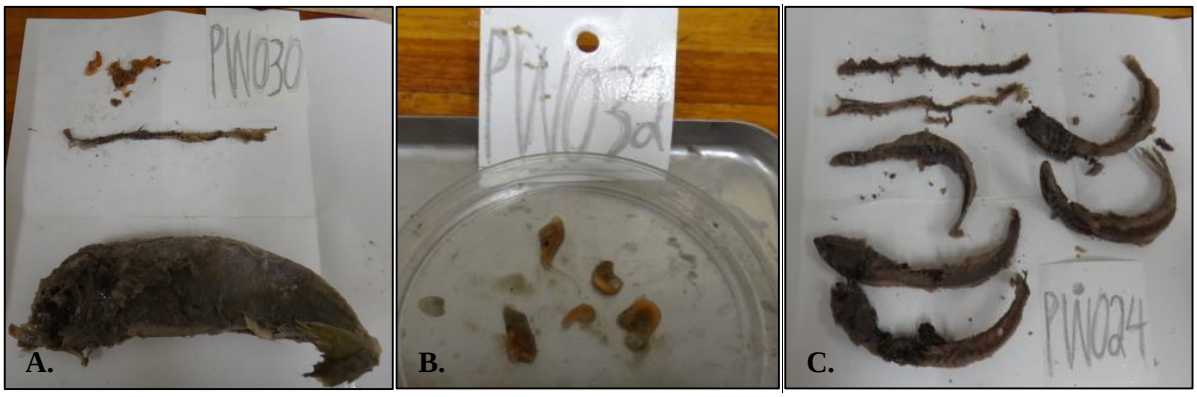

Figure 3.17 Photographs of the stomach contents of *Pliotrema warreni* including the smallest and largest individuals in the sample and the range of states of the fish vertebrae. A.) Cape horse mackerel, a round herring vertebrae, and bits from at least three shrimp. B.) Contents of the smallest individual's stomach which contained only five small shrimp. C.) Range of states of the fish vertebrae recovered from the stomachs.

Five small shrimp of an unidentified species were found in the stomach of the smallest male. Remains of shrimp were also found in the stomachs of two other larger females. Making a definitive identification of the fish species became much more difficult without a head region to examine as all the heads had been crushed and digested to a point of indiscernibility (Fig. 3.17). The near completely digested shrimp bits made identification difficult. The fish vertebrae make up over half of the contents (55.47%). Much of the solid material was bits of fish meat and a large portion of the liquid was of some gelatinous substance of unknown origin (Fig. 3.18). The solid contents are further examined by percent occurrence within the stomachs (Fig. 3.19)

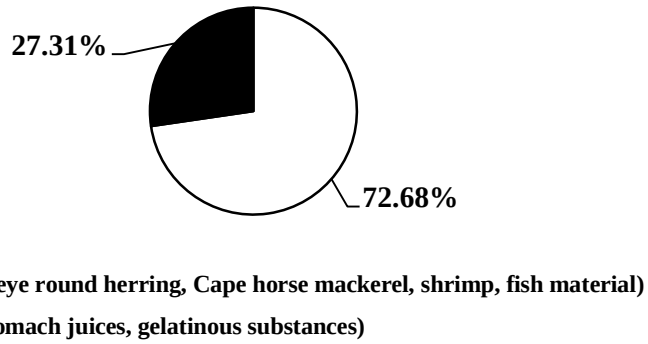

Figure 3.18 Percent composition of items in stomach of *Pliotrema warreni* denoting that the solid contents consisting of the vertebrae of redeye round herring, Cape horse mackerel, digested shrimp, and heavily digested fish material comprised nearly ¾ of the contents by mass present in the stomachs with the remaining gastric juices, unknown gelatinous substances, and residual formalin from preservation comprising just over the remaining ¼ of the stomach contents

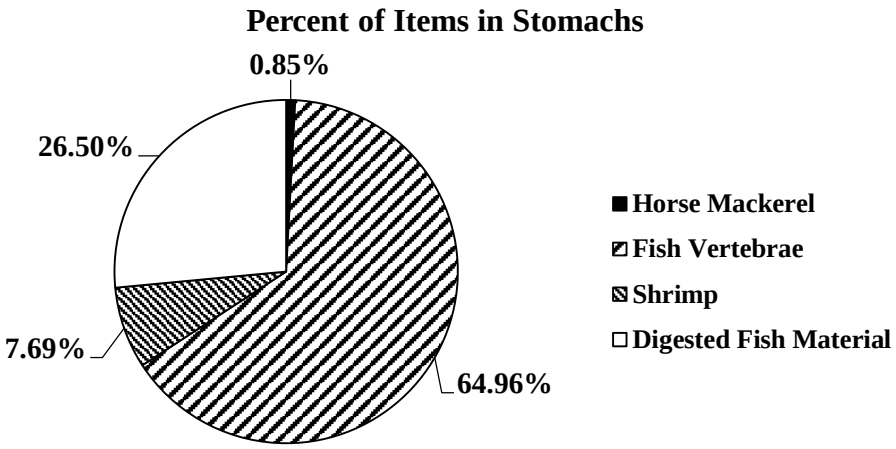

Figure 3.19 Percent occurrence of items present in stomach content analysis showing the importance of redeye round herring (64.96%) in the diets of *Pliotrema warreni*

The number of fish vertebrae found in the stomachs increased with the total length of the shark (p-value = 0.0356, $R^2 = 0.14$) (Fig. 3.20). The mean length of vertebral columns were 93.86 mm which is within the size range of redeye round herring, *Etrumeus whiteheadi*, which can reach 200 mm total length (Pillar & Barange 1998). However, the relationship between the lengths of the fish vertebrae and the total length of the individual shows no correlation at all (Fig. 3.21).

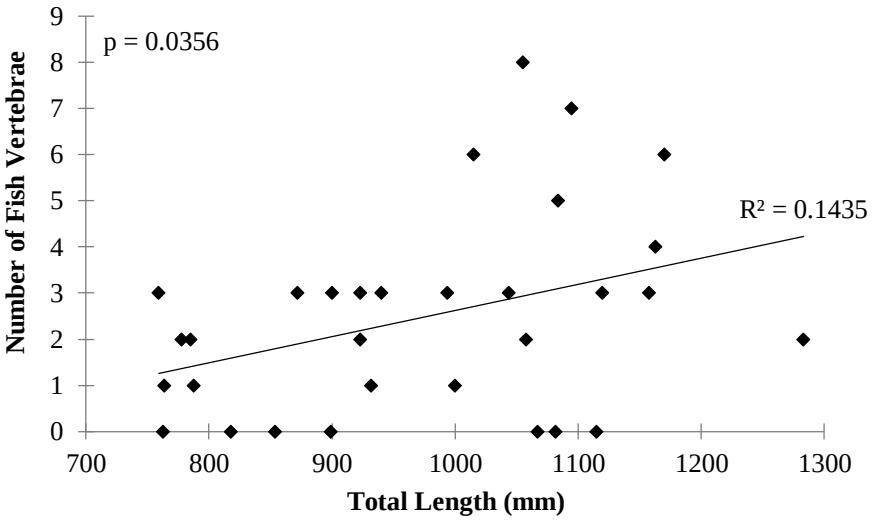

Figure 3.20 The relationship between number of fish vertebrae in the stomachs and total length of *Pliotrema warreni*

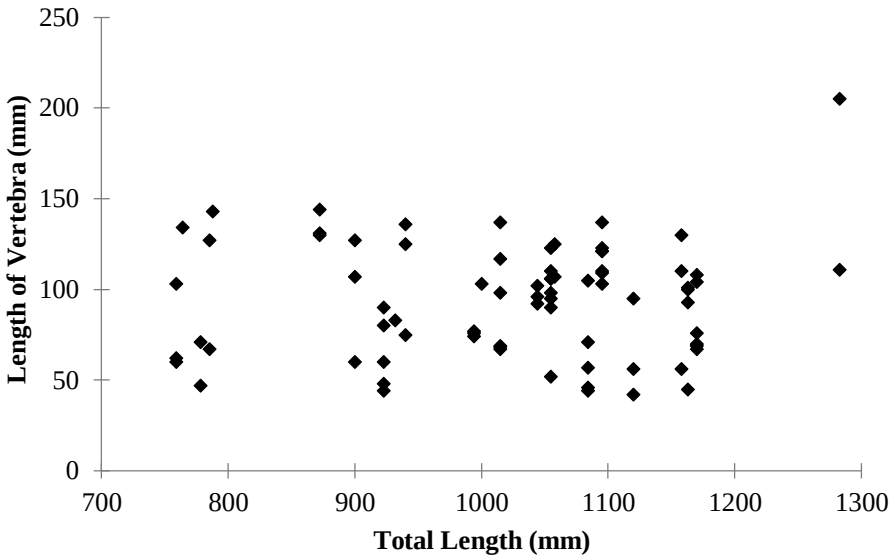

Figure 3.21 A scatterplot of the length of fish vertebrae and total length of *Pliotrema warreni*

3.3 Parasite Assessment

Twenty-three individuals across three parasitic phyla were collected from the 32 sharks examined.

Table 3.6 Prevalence (%), mean intensity, and relative abundance of the four parasites found in *Pliotrema warreni.*

Parasite	Group	No. Examined	No. Infected	% Prevalence	No. Parasites	Mean Intensity	% Abundance
Nematodes *(Proleptus obtusus)*	Male	7	4	57.14	4	1.00	57.14
	Female	25	7	28.00	11	1.57	44.00
	Combined	32	11	34.38	15	1.36	46.88
Nematodes ***(****Ascaris* sp.*)***	Male	7	0	0.00	0	0.00	0.00
	Female	25	2	8.00	2	1.00	8.00
	Combined	32	2	6.25	2	1.00	6.25
Acanthocephalans ***(****Neoechinorhynchidae)***	Male	7	0	0.00	0	0.00	0.00
	Female	25	2	8.00	3	1.50	12.00
	Combined	32	2	6.25	3	1.50	9.38
Isopods *(Cymothidae)*	Male	7	0	0.00	0	0.00	0.00
	Female	25	1	4.00	3	3.00	12.00
	Combined	32	1	3.13	3	3.00	9.38

Figure 3.22 Parasites of *Pliotrema warreni*: (A) Photograph of cymothoid species of isopod found on the external surface of one shark specimen between the first dorsal and left pectoral fins. (B) Species of acanthocephalan of the family Neoechinorhynchidae found in stomach of two specimens. (C) *Ascaris* sp. nematode found in the body cavity around the stomach of one specimen. (D) *Proleptus obtusus* nematodes found in the stomachs of numerous specimens.

The nematode, *Proleptus obtusus* (Fig. 3.22D), from the stomachs of 11 sharks were most abundant with a prevalence of 34.38% and abundance of 46.88%. Isopods of the family Cymothoidae (Fig. 3.22A) found on the external surface of the body had an overall prevalence of 3.13% and abundance of 9.38% (Table 3.6). Acanthocephalans of the family Neoechinorhynchidae (Fig. 3.22B) were found in the stomachs with a prevalence of 6.25% and abundance of 9.38% over all the hosts examined. Nematodes of the genus *Ascaris* (Fig. 3.22C) were collected from the visceral cavity with a prevalence of 6.25% and abundance of 6.25%. No relationship between total length and mean parasite intensity existed in this species (Fig. 3.23).

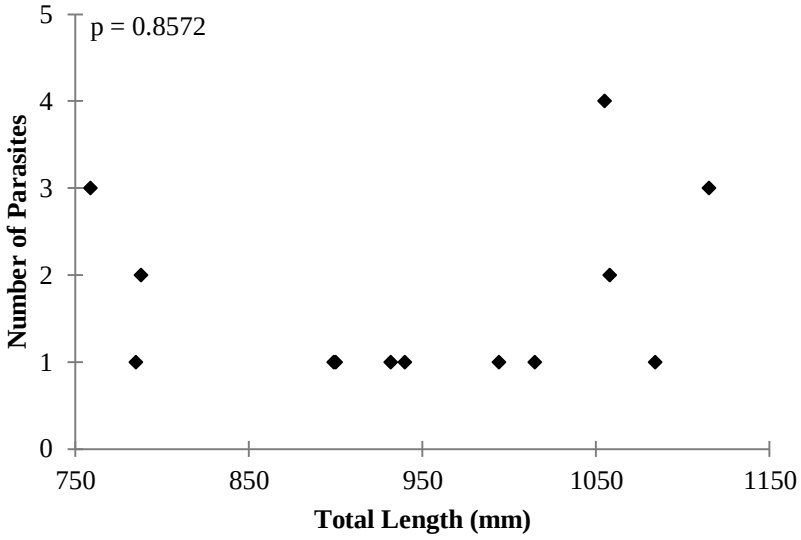

Figure 3.23 The relationship between the number of parasites present and the total length of *Pliotrema warreni*

Endoparasites composed a 43.75% prevalence and a 62.50% abundance in total whilst ectoparasites composed a 3.13% prevalence and a 9.38% abundance.

Table 3.7 Prevalence (%), mean intensity, and relative abundance of the parasites found internally and externally on *Pliotrema warreni*.

Location	Group	No. Examined	No. Infected	% Prevalence	No. Parasites	Mean Intensity	% Abundance
Ectoparasite	Male	7	0	0.00	0	0.00	0.00
	Female	25	1	4.00	3	3.00	12.00
	Combined	32	1	3.13	3	3.00	9.38
Endoparasite	Male	7	4	57.14	4	1.00	57.14
	Female	25	10	40.00	16	2.10	84.00
	Combined	32	14	43.75	20	1.43	62.50

3.4 Vertebral Age Determination

The ages of these specimens could not be determined. The only visible growth rings were the initial angle change, or birthmark. This denotes the point at which the individual changes from yolk in the womb to a self-sustaining diet in the ocean after birth. Subsequent growth rings that may have been laid down after this angle change were not visible as the dye had not adhered to it or it had broken down completely.

Chapter 4: DISCUSSION:

The majority of the published information on *P. warreni* comes from just a few publications by only a few biologists (Compagno, 1999; Fowler, 2004; Compagno *et al.*, 2005; Ebert & Cailliet, 2011; Ebert & Wilms, 2013; Ebert *et al.*, 2013; Ebert & van Hees, 2015). The Australian sawshark, *Pristiophorus cirratus*, species is the most studied sawshark (McKenzie & Caira, 1998; Compagno, 1999; Compagno *et al.*, 2005; Ebert *et al.*, 2013). The reason for such a lack of information is likely that sawshark species are mostly bycatch species. The IUCN lists fishing and bycatch as a threat to nearly all sawshark species (Ebert *et al.*, 2013). However, changes in management and the adoption of an ecosystem approach to fisheries will allow for more knowledge to be gathered on deep water and bycatch species.

4.1 Morphometrics

Ebert *et al.* (2013) reports the *Pliotrema warreni* are born at a length between 35 and 37 cm. While there is no record of how long the 32 sharks in this study were at birth, the smallest female was 75.9 cm and the smallest male 76.3 cm. The same literature also showed that the maximum lengths of this species are 136 cm for females and 112 cm for males. While this study used a fairly small sample size, the largest female was recorded as 128.3 cm and the largest male at 101.5 cm, neither of which are higher than the reported maximum length for either sex. The results of this study show that males and females fall within the reported length spectrum for the species. They are the third largest sawshark species behind *Pristiophorus cirratus* and *P. japonicus* (Ebert *et al.*, 2013). Males and females were determined to have different average total lengths.

Females became larger in girth as they grew longer while males did not display as noticeable a change. The differentiation between male and female correlations between girth and total length could largely be influenced by maturity state of the individual and/or by the amount of food in the stomach. Fuller stomachs will occupy more space within the body cavity. Pregnant females will be housing embryos and gaining weight to provide for the offspring. Males and females were determined to have different average mass. However, despite differences in mean length and mass between sexes, the mean organ mass for heart and stomach were almost identical and the average mass for livers were within 2% of each other. These data show the organ masses do not vary by sex.

Females are said by Ebert *et al.* (2013) to reach sexual maturity at 110 cm. This study revealed that females shorter than 100 cm were sexually immature and those over 100 cm were mature. Sexual maturity was indicated in these females by the presence of a widened uterus. If the uterus had widened, it was marked as sexually mature and further measurements were taken. These data in this study conclude that females reach sexual maturity by 100 cm which is 10 cm shorter than reported in Ebert *et al.*, (2013). Of the 25 females in this study, 14 were found to be sexually mature, all of which were 100cm or greater in total length. From the 14 mature females, the ovaries were inspected for fertilized eggs which were greater than 10 mm in diameter. Only two of the 14 individuals had any eggs which were of this size or greater. The very first female dissected (PW001) had four eggs greater than this size in its right ovary and eight eggs in its left ovary. The second to last female (PW030) also had these large fertilized eggs with six in the left ovary and eight in the right.

Ebert *et al.*, (2013) reports that males mature at 83 cm. The results of the analysis of the seven males in this study, all males above 83 cm (4 total) demonstrated claspers in a stage of either 2 or 3 and which were capable of moderate or complete articulation. Clasper state 2 indicated that there was articulation capabilities, however, such claspers did not have the complete range of motion. State 3 had full articulation, i.e. a total 360 degree range of motion. The three males under 83 cm in length did not have claspers with the articulation capability and were marked at a clasper state 1. One male at a total length of 78.5 cm had sperm present even though he was not at a length long enough to be considered sexually mature. The remaining two sexually immature males were of shorter total lengths and also did not have any sperm presence, further confirming their sexual immaturity.

Given that in this sample size more than half of both the males and females are sexually mature, the area around Bird Island in Algoa Bay on the Eastern Cape of South Africa could be an potential area where mating occurs. The trawl survey which collected the individuals used in this study was conducted in the waters just outside of the no-take zone around Bird Island in Algoa Bay. Ebert *et al.*, (2013) says that this species occurs from 37-500 m depth and is speculated to move closer inshore in order to give birth at specific pupping grounds. The location from which this sample was collected happen to be at depths ranging from 50-120 m, which is inshore of the deeper 200-500 m depths in which this species can be found.

Average length does not differ between the sexes. The lengths measured in this study positively correlated with total length and demonstrated that this species grows

hypoallometrically. Average mass does differ with females being statistically heavier than males. There was no significant difference in heart and stomach masses between sexes, however, liver mass did differ significantly with females having the heavier liver. No studies have been conducted to determine if other sawsharks share these growth characteristic. However, one study by Karim *et al.*, (2013) compared growth rates of a variety of sharks, skates, and rays to determine whether the growth patterns for each were isometric, positive allometric or negative allometric growth. One species of sawfish, *Pristis cuspidatus*, was shown to grow isometrically while the only other sharks to demonstrate this were the tiger and silky sharks. Six of the 13 ray species surveyed displayed isometric growth, the most of all three groups of elasmobranchs surveyed.

In regards to rostrum length, for both males and females, the correlation between rostrum length and total length is quite strong below 100 cm total length. No male specimens exceeded this length, and all females were indicated as sexually mature. The slightly weaker correlation above this length could indicate that as females reach sexual maturity more energy and effort goes into procreating and providing for offspring and, therefore, leaves less energy to be invested in growing out their rostrum.

The findings of this study revealed that there are roughly 176 rostral teeth. Typically there are one to three smaller teeth between each larger one. The barbels are located closer to the mouth on this species than most other sawsharks and the rostral teeth extend back to just behind the mouth (Fig. 4.1). There is a negative correlation between rostrum length and number of rostral teeth, contrary to the expectation. As discussed by Slaughter & Springer (1968) sawfish rostral teeth are set at a certain number for their lifetime and any teeth that are lost are, therefore, not replaced. However, in sawsharks, they maintain, that the rostral teeth are replaced as the shark grows older. Slaughter & Springer (1968) denote in their findings that sawsharks are born with their rostral teeth fully formed and covered by a membrane. This membrane is worn away shortly after birth and throughout the younger years of the sawsharks they cycle through three sets of rostral teeth. As with all the other species of sawshark, there are typically large and smaller rostral teeth (Slaughter & Springer 1968). The reasons for the trend supporting fewer teeth with increasing rostrum length could be attributed to more wear and tear on the rostral teeth from daily activities and damage as the shark gets older and larger. Further study into the relationship between rostral tooth number and growth need be conducted on a larger sample size and across equal numbers of male and female specimens.

Figure 4.1 Rostrum of *Pliotrema warreni* with large teeth showing one to three smaller teeth in between as well as rostral teeth extending just behind the mouth

4.2 Stomach Content Analysis

A large majority of the prey items discovered in the gut contents were determined to be redeye round herring, *Etrumeus whiteheadi*. Whilst most of the remains were mostly digested and left only vertebrae, some remains were intact enough to make a positive identification. Fish that were still in a less digested state had the heads crushed and digested. This consistent trend of crushed heads on all the fish could may well be an indication of how this species feeds. Ebert *et al.*, (2013) suggests that sawsharks use the ampullae of Lorenzini on the underside of the rostrum to locate prey which they stun with the rostrum. The crushed heads of prey could be due to the way the sawsharks eat the prey after it has been stunned. One female specimen also had a large Cape horse mackerel, *Trachurus trachurus capensis*, in the stomach.

Redeye round herring, *Etrumeus whiteheadi* and Cape horse mackerel, *Trachurus trachurus capensis* are common around the Bird Island area of Algoa Bay. These species also demonstrate vertical migrations where they feed on the bottom in the morning to mid-day and then migrate up the water column later in the day and into the night (Pillar & Barange 1998). Also, knowing that bottom trawlers in South African targeting sardines and anchovy frequently encounter redeye and Cape horse mackerel as bycatch. Given that these sharks feed on or near the bottom, this increases the likelihood in which to feed on these particular species of fish. There were also several species of decapod shrimp that also play a role in the diet of *P. warreni*. These small crustaceans and other small pelagic fish are the key components in the diet of *P. warreni*.

Ebert *et al.* (2013) note that this species also predates on squid, though no clear remains of squid were found in the stomachs aside from the occasional gelatinous mass that might have once been part of a squid and was by then heavily digested. Since the shrimp were only found in two individuals and one tiny individual had what looked to be a different species of shrimp,

it may well be that shrimp are much scarcer throughout the range of this shark and, therefore, do not play as crucial of a role in the diet or predominantly a prey item reserved for smaller individuals as there were five shrimp in the stomach of the smallest shark in the survey. There was no distinguishing difference in stomach mass between sexes nor was there any discernable differences in stomach content which might denote a sex-linked dietary preference.

4.3 Parasite Assessment

The macroparasite survey of *P. warreni* did not reveal a high level of diversity or abundance of parasites. As with a vast majority of benthic and epibenthic species of fish and elasmobranchs, parasites are quite a common occurrence. However, in these sharks there were surprisingly few. The reason might be the area from which these sharks were captured, or perchance these particular sharks have a rather strong immune system. One shark out of 32 was found to be infected by isopods from the family Cymothoidae (Kensley 1978) located behind the pelvic fin and below the first dorsal fin. All other parasites were sampled from inside the body cavity or within the stomach. Two species of nematode which were found infecting the visceral cavity and stomach were identified as *Ascaris* sp. and *Proleptus obtusus*. A species of acanthocephalan from the family Neoechinorhynchidae (Amin 2002) was also found infecting the stomach. Beyond these parasites *P. warreni* harbored no similar parasite species as have been found in other sawsharks, likely due to geographical isolation and differences in diet.

This study revealed four parasite species. The two nematode species previously identified by Yeld (2009) and Morris (2015) were also found in *P. warreni*. This study found the nematode *Proleptus obtusus* to be 34.4% prevalent. This is far less prevalent than Yeld (2009) found in the three shyshark species where all had 100% prevalence of *P. obtusus*. Morris (2015) found two species of sandshark to have a *P. obtusus* prevalence of 31.6% in *Rhinobatos annulatus* and 29.4% in *Rhinobatos blochii*, both of which are slightly lower than that found in *P. warreni*. Morris (2015) further found the nematode *Ascaris* sp. to be 11.8% prevalent while this study found that of P. warreni to be slightly lower at 6.3% prevalence.

All of the other parasites found in the organs and blood of other South African elasmobranch species were not found in *P. warreni*. Due to being frozen prior to examination the organs and blood were rendered useless in a parasite survey for microparasites. However, two species of parasite previously undocumented in *P. warreni* were found. The cymothid isopods found on

one female had a 3.1% prevalence. These isopods differ from those found by both Yeld (2009) and Morris (2015) in the family from which they belong, taxonomically. The neoechinorhynchid acanthocephalans found in two females had a 6.3% prevalence. Acanthocephalans are currently undocumented in elasmobranchs. The difference in types of parasites found in *P. warreni* compared to other surveyed species stems from differences in geographic locations as well as variances in diets.

This is the first survey of parasites infecting *P. warreni*. Further identification to the species level of the isopod and acanthocephalans found in this study is required. Of the seven males sampled 57.14% were host to at least one parasite while 76% of the females sampled had at least one parasite; thus males and females did not have the same number of parasites nor do they have the same species of parasites. However, this trend can likely be attributed to the higher abundance of female sharks in the study than males. Females were also found to have more parasites and a greater diversity of parasites than males. The prediction that intensity of infection by parasites increases with size was also found incorrect. There was virtually no relationship between intensity of parasites and total length.

4.4 Vertebral Age Determination

In the current literature, only one species of sawshark has been aged and the results of those aging procedures are tentative at best. Ebert *et al.* (2013) reports that the shortnose or Southern sawshark, *Pristiophorus nudipinnis*, has been aged or at least reported to live to a maximum age of nine years. Aging sharks is complicated as their vertebrae, as with all other parts of their skeleton, are made of cartilage. A number of different techniques have been developed with the intention of determining ages of various elasmobranch species as well as aiding in determining aspects pertaining to their life histories and reproductive traits. One fairly successful way to age certain sharks is to stain the vertebral centrum with silver nitrate which impregnates the calcified rings and causes them to appear dark under an ultraviolet light (Wischniowski 2006). Other frequently used methods include staining the centra with various concentrations of aniline blue, alizarin red, and crystal violet, all of which are aimed at having the dyes adhere to the calcified age rings within the vertebral centra (Wischniowski 2006). A final and more intensive and expensive technique is taking x-radiographs of the centra (Kusher *et al.*, 1981). Many other methods include soaking the centra in various concentrations of 3% hydrogen peroxide, sodium hydroxide, xylene, 95% and 100% isopropyl alcohol, formalin, formic acid, and both 70% and 100% alcohol individually or a combination of a number of

these things. Another method has been developed by which the neural arches of the vertebrae are stained with silver nitrate with a similar intention as the silver nitrate staining of the vertebral centra itself (McFarlane *et al.*, 2002). All of these methods were designed with the aim of expounding the circuli in the centra in order to count them to determine the age of the elasmobranch in question (Kusher *et al.*, 1981).

After the centra have been cleaned, the procedure used to count the rings must be well explained in order to be reproducible in future studies and by other researchers in the current study (Kusher *et al.*, 1981). When, or rather if, whatever technique used works correctly and the calcified circuli are clearly revealed then the process of counting them and determining the age of the elasmobranch in question can begin. Even if all of the aforementioned procedures go as planned there are still problems that can arise from samples being dried. During many of these drying procedures between washing and staining, the centra are subject to cracking and shrinking which can further lead to problems reading the circuli (Wischniowski 2006). Many deep water shark (prickly shark, *Echinorhinus cookei*, brown cat shark, *Apristurus brunneus*, filetail catshark, *Parmaturus xaniurus*) as well as primitive sharks (sevengill shark, *Notorhynchus cepedians*, bluntnose sixgill shark, *Hexanchus griseus*, Pacific sleeper shark, *Somniosus pacificus*) have proven incredibly difficult to age and to date have not successfully been aged (Wischniowski 2006). The same problems that have created issues with aging these particular sharks are the same issues that plagued the research in this thesis and caused the extensive preparation to fail for *Pliotrema warreni*.

The main cause for this frustration is the relatively low levels of calcium and phosphate deposits within the vertebral centrum. Without these deposits, the stains have nothing to adhere to and, therefore, no circuli are clearly visible with which to determine ages of whatever elasmobranch is in question (Wischniowski 2006). This was the ultimately problem with aging the sixgill sawshark with the only distinguishable markings that took the stain was the initial angle change which indicates birth of the individual and a change from a diet of yolk from the yolk sac in the womb to a diet of what has been presumed to be small fish and small crustaceans. New techniques will need to be developed to age these and many other sawsharks. The result of all of this proved the final hypothesis neither correct nor incorrect, but rather inconclusive given that the ages of these sharks could not be determined. As for the final hypothesis that males and females are the same ages at the same length and mass measurements falls inconclusive since no aging could successfully be accomplished.

4.5 Conclusions

This study goes on record to show how much more information there is yet to be determined about *Pliotrema warreni*. This is also the first in depth survey of macroparasites infecting *P. warreni* in southern Africa. A new species of acanthocephalan formerly undocumented in elasmobranchs was found. The acanthocephalan along with the isopod require further examination for taxonomic purposes for addition to the known biodiversity of southern African marine ecosystems. Better understanding the host as well as the parasites infecting it serve to help ensure that both host and parasite continue to be players in the overall ecosystems for generations to come. A greater understanding of the parasites infecting the *P. warreni* as well as its biology allow for a greater success in preserving *P. warreni* and moving it to an IUCN Red List 'Least Concern' status in the future.

The results of this study show that females are both longer in total body length and heavier in overall body mass than males, though both sexes grow hypoallometrically. Females reached maturity at 1000 mm while males reached sexual maturity at 850 mm. Prey items were predominantly small fish that display diurnal vertical migration patterns. Conventional methods proved ineffective for vertebral age determination. Four different parasite species were found during the examinations. Two were species of nematode, one was an isopod and the last was a species of acanthocephalan previously unidentified in elasmobranchs. The results of this study show that there is far more research that need be completed to better understand *Pliotrema warreni* and not only the role it plays in the southern African marine ecosystem but also as an IUCN Red List 'Near Threatened' species.

Appendix

Table 1. Partial data set collected during research and dissection. Females are white, males are highlighted in green; the two in yellow were collected from a separate cruise at a different site.

Sample Number	Mass (g)	Total Length (mm)	Standard Length (mm)	Girth (mm)	Rostrum Length (mm)	Number of Rostral Teeth	IO-PC (mm)	DO-PC (mm)	DO1-DO2 (mm)	Mouth Width (mm)
PW001	2839	1055	856	299	N/A	N/A	638	400	240	50
PW002	602.5	759	620	158	207	232	414	259	157	35
PW003	671	778	640	165	211	176	428	273	165	35
PW004	1273	923	745	213	240	176	520	330	200	41
PW005	895.5	818	680	183	227	188	460	290	183	36
PW006	1845.5	1082	881	230	279	194	610	390	243	46
PW007	1258.5	900	740	195	237	159	503	326	205	44
PW008	2347.5	1120	927	247	302	153	621	395	252	53
PW009	1804.5	1044	868	221	276	182	591	375	232	49
PW010	2541	1170	967	257	286	176	677	442	274	54
PW011	2117.5	1084	893	236	283	180	606	382	230	46
PW012	1747.5	1000	813	228	255	154	565	355	213	46
PW013	1903.5	1058	880	216	270	177	613	382	225	45
PW014	1090	872	720	183	241	173	480	302	178	39
PW015	612.5	764	623	144	215	220	413	263	163	37
PW016	1158.5	940	770	180	253	167	521	328	207	43
PW017	747.5	785	640	172	206	169	438	285	175	33
PW018	2589.5	1158	964	284	310	163	660	410	255	54
PW019	1282	932	772	204	259	167	517	330	192	42
PW020	1327.5	923	755	207	256	143	500	320	190	42
PW021	1187	899	733	194	243	187	493	310	190	40
PW022	1592.5	1015	851	214	268	178	581	378	233	45
PW023	1756	1067	884	216	291	187	588	366	225	49

PW024	1970	1095	900	213	275	178	624	344	243	50
PW025	791.5	788	653	175	217	174	442	289	172	38
PW026	1593.5	994	820	213	259	156	567	362	220	42
PW027	1016.5	854	702	190	230	171	478	300	190	37
PW028	2396	1163	960	231	294	155	665	435	265	51
PW029	687	763	630	156	213	198	420	267	158	32
PW030	3478.5	1283	1060	299	334	141	718	450	281	55
PW031	2234.5	1115	922	248	290	201	634	403	250	46
PW032	N/A	N/A	N/A	125	N/A	N/A	333	207	130	25

Table 1. Description of important internal organ mass measurements.

Sample Number	Liver Color	Liver Mass (g)	Heart Mass (g)	Stomach Mass (g)
PW001	Beige with some black splotches	263.5	2	94
PW002	Beige	36	0.5	16.5
PW003	Beige	49	0.5	23.5
PW004	Beige with blackend ends	125	1.5	34.5
PW005	Beige	76	1	21.5
PW006	Beige	167.5	1.5	32
PW007	Beige with blackend ends	94	1	52.5
PW008	Beige with a bit of black streaks	167	1.5	69
PW009	Beige	154.5	1.5	59
PW010	Beige with some black splotches	271	2.5	87.5
PW011	Beige	91.5	1	66.5
PW012	Beige with a bit of black streaks	144.5	1.5	94.5
PW013	Beige with clotted blood	129	0.5	71
PW014	Beige	59.5	0.5	66
PW015	Beige	36	0.5	39
PW016	Beige with some black streaks	58	1	73

PW017	Beige	39	0.5	43
PW018	Beige	205	2	108
PW019	Beige with grey ends	123	1	35.5
PW020	Beige with black streaks	110.5	1	48.5
PW021	Beige (best looking one so far)	102	1	30.5
PW022	Beige	104	2	77
PW023	Beige	125	2	37
PW024	Beige	16.5	1.5	125.5
PW025	Beige	64.5	0.5	44.5
PW026	Beige	92.5	2	16.5
PW027	Beige	81	1	27
PW028	Beige	232	2	77
PW029	Beige	50	1	19
PW030	Beige	394	3	173.5
PW031	Beige	224	2	36.5
PW032	Beige	19	0.5	11.5

Table 2. Description of all female morphometric data taken during the project.

Sample Number	Maturity	Number of Eggs > 10mm Right	Number of Eggs > 10mm Left	Ovary Mass Right (g)	Ovary Mass Left (g)	Ovary Mass Total (g)	Oviducal Gland Length Right (mm)	Oviducal Gland Length Left (mm)	Oviducal Gland Width Right (mm)	Oviducal Gland Width Left (mm)	Uterus Widened	Uterus Mass Right (g)	Uterus Mass Left (g)	Uterus Mass Total (g)
PW001	Mature	4	8	215	250	465	20	20	140	150	Yes	75	75	150
PW002	Immature	0	0	N/A	N/A	15	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW003	Immature	0	0	N/A	N/A	0.5	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW004	Immature	0	0	N/A	N/A	1	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW005	Immature	0	0	N/A	N/A	9.5	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW006	Mature	0	0	9.5	12	21.5	15	15	10	10	Yes	6	6	12

PW007	Immature	0	0	N/A	N/A	2	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW008	Mature	0	0	9	11	20	15	16	13	14	Yes	10.5	10.5	21
PW009	Mature	0	0	35	35	70	20	15	12	13	Yes	8	7.5	15.5
PW010	Mature	0	0	9.5	5.5	15	18	18	12	12	Yes	12	12.5	24.5
PW011	Mature	0	0	7.5	10	17.5	17	16	13	11	Yes	10.5	9	19.5
PW012	Mature	0	0	9.5	6.5	16	13	14	8	10	Yes	5	5	10
PW013	Mature	0	0	2.5	1.5	4	16	14	10	13	Yes	8	8	16
PW014	Immature	0	0	N/A	N/A	0.5	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW015	Immature	0	0	4.5	5	9.5	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW018	Mature	0	0	15	15.5	30.5	15	17	10	10	Yes	9	9.5	18.5
PW019	Immature	0	0	6	4.5	10.5	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW020	Immature	0	0	7.5	5	12.5	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW021	Immature	0	0	10	9	19	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW023	Mature	0	0	13.5	10	23.5	9	9	9	9	Yes	6	6	12
PW024	Mature	0	0	12.5	13	25.5	19	18	11	10	Yes	10	11	21
PW025	Immature	0	0	4.5	5.5	10	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
PW028	Mature	0	0	5.5	4	9.5	22	18	12	10	Yes	10.5	9.5	20
PW030	Mature	6	8	58	77.5	135.5	17	20	13	15	Yes	17.5	16.5	34
PW031	Mature	0	0	6.5	7.5	14	15	14	10	10	Yes	10	11.5	21.5

Table 3. Description of all male morphometric data collected during the project.

Sample Number	Clasper State	Clasper Length Right (mm)	Clasper Length Left (mm)	Articulation	Sperm	Testes Mass Right (g)	Testes Mass Left (g)	Testes Mass Total (g)
PW016	3	71	68	Yes	Yes	5	8.5	13.5
PW017	1	39	40	No	Yes	5	5	10
PW022	2	56	56	Yes	Yes	9.5	8	17.5
PW026	2	65	63	Yes	Yes	8.5	9.5	18
PW027	2	58	57	Yes	Yes	3.5	3.5	7

PW029	1	16	16	No	No	1	2	3
PW032	1	10	10	No	No	N/A	N/A	N/A

Table 4. All recorded stomach data collected during the stomach content analysis portion of the project.

Sample Number	Solid Stomach Content Mass (g)	Liquid Stomach Content Mass (g)	Total Stomach Content Mass (g)	Stomach Contents	Number of Fish Vertebrae	Stage (1-3)	Length (mm)	Stomach Parasites	Number of Stomach Parasites
PW001	39	7.5	46.5	Fish/Miscellaneous Mush	8	2	52, 90, 95, 98, 106, 106, 110, 123	Nematode & Acanthocephalan	4
PW002	2	1	3	Fish/Miscellaneous Mush	3	2	60, 62, 103	Nematode	3
PW003	6	0	6	Fish/Miscellaneous Mush (Maybe Squid)?	2	2	47, 71	N/A	0
PW004	6.5	6.5	13	Fish/Miscellaneous Mush	3	2	48, 80, 90	N/A	0
PW005	5.5	3	8.5	Fish Mush (No Vertebrae)	0	3	N/A	N/A	0
PW006	3	0	3	Fish Mush (No Vertebrae)	0	3	N/A	N/A	0
PW007	12.5	8.5	21	Fish/Miscellaneous Mush	3	2	60, 107, 127	Nematode	1
PW008	19	5.5	24.5	Fish/Miscellaneous Mush	3	2	42, 56, 95	N/A	0
PW009	16.5	16.5	33	Fish/Miscellaneous Mush	3	2	92, 96, 102	N/A	0
PW010	17.5	26	43.5	Fish/Miscellaneous Mush	6	2	67, 69, 70, 76, 104, 108	N/A	0
PW011	26	5	31	Fish/Miscellaneous Mush	5	2	44, 46, 57, 71, 105	Nematode	1
PW012	14	32	46	Fish/Miscellaneous Mush	1	2	103	N/A	0
PW013	18.5	18	36.5	Fish/Miscellaneous Mush	2	2	107, 125	Acanthocephalan	2
PW014	36.5	9	45.5	Fish/Miscellaneous Mush	3	1	130, 131, 144	N/A	0
PW015	20	0	20	Fish/Miscellaneous Mush	1	1	134	N/A	0
PW016	27	20.5	47.5	Fish/Miscellaneous Mush	3	2	75, 125, 136	Nematode	1

PW017	23	4.5	27.5	Fish/Miscellaneous Mush	2	2	67, 127	Nematode	1
PW018	32.5	26.5	59	Fish/Miscellaneous Mush	3	2	56, 110, 130	N/A	0
PW019	13	0	13	Fish/Miscellaneous Mush/Shrimp Head	1	3	83	Nematode	1
PW020	16.5	6	22.5	Fish/Miscellaneous Mush	2	2	44, 60	N/A	0
PW021	3.5	0	3.5	Fish Mush (No Vertebrae)	0	3	N/A	Nematode	1
PW022	37	17.5	54.5	Fish/Miscellaneous Mush	6	2	67, 68, 69, 98, 117, 137	Nematode	1
PW023	4	0	4	Fish Mush (No Vertebrae)	0	2	N/A	N/A	0
PW024	73	6	79	Fish/Miscellaneous Mush	7	2	103, 109, 110, 121, 121, 123, 137	N/A	0
PW025	23.5	3.5	57	Fish/Miscellaneous Mush	1	2	143	N/A	0
PW026	8	10	18	Fish/Miscellaneous Mush	3	2	74, 76, 77	Nematode	1
PW027	10.5	0	10.5	Fish Mush (No Vertebrae)	0	3	N/A	N/A	0
PW028	22	22	44	Fish/Miscellaneous Mush	4	2	45, 93, 100, 101	Nematode	1
PW029	7	0	7	Fish Mush (No Vertebrae)	0	3	N/A	N/A	0
PW030	126	0	126	Fish/Miscellaneous Mush/Shrimp Bits	2	2	111, 205	N/A	0
PW031	3.5	0	3.5	Fish Mush (No Vertebrae)	0	3	N/A	N/A	0
PW032	6	0	6	Full Shrimp	0	1	N/A	N/A	0

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